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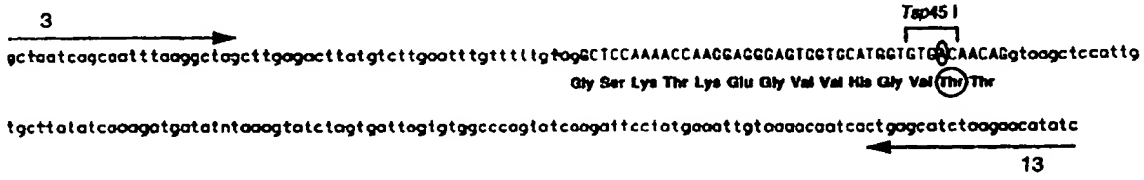

(57) Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2 percent. It was recently reported that a PD susceptibility gene is located on the long arm of human chromosome four. The present invention reports the subsequent identification of a mutation in the alpha synuclein gene, which codes for a presynaptic protein thought to be involved in neuronal plasticity. The finding of a specific molecular alteration which is causative for PD will permit the detailed understanding of the pathophysiology of the disorder, which will lead to potential therapeutic interventions, as well as a means for diagnosing individuals having an increased risk of developing the disease.

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CLONING OF A GENE MUTATION FOR PARKINSON'S DISEASE

BACKGROUND OF THE INVENTION

1. Field of the Invention

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2 percent. A pattern of familial aggregation has been documented for the disorder, and it was recently reported that a PD susceptibility gene in a large Italian kindred is located on the long arm of human chromosome 4. We have identified a mutation in the alpha synuclein gene, which codes for a presynaptic protein thought to be involved in neuronal plasticity, in the Italian kindred and in three unrelated families of Greek origin with autosomal dominant inheritance for the PD phenotype. This finding of a specific molecular alteration which is causative for PD will permit the detailed understanding of the pathophysiology of the disorder. In addition, methods of screening nucleic acids for the presence of mutations in the synuclein gene to test for predisposition to Parkinson's Disease are now possible.

2. Technology Background

Parkinson's disease (PD) was first described by James Parkinson in 1817 (*1*). The clinical manifestations of this

neurodegenerative disorder include resting tremor, muscular rigidity, bradykinesia and postural instability. A relatively specific pathological feature accompanying the neuronal degeneration is the intracytoplasmic inclusion body, known as the Lewy body, which is found in many regions including the substantia nigra, locus ceruleus, nucleus basalis, hypothalamus, cerebral cortex, cranial nerve motor nuclei, and the central and peripheral divisions of the autonomic nervous system (*1*).

In many cases a heritable factor predisposes to the development of the clinical syndrome (*2*). We have recently shown that genetic markers on human chromosome 4q21-q23 segregate with the PD phenotype in a large family of Italian descent (*3*). The clinical picture of the PD phenotype in the Italian kindred has been well documented to be typical for PD, including Lewy bodies, with the exception of a relatively earlier age of onset of illness at 46 ± 13 years. In this family the penetrance of the gene has been estimated to be 85%, suggesting that a single gene defect is sufficient to determine the PD phenotype.

We now report the identification of a mutation in the alpha synuclein gene that is associated with Parkinson's disease. The mutation, an Ala53Thr substitution, was found to be linked to the PD phenotype in four independent PD families and absent from 314 control chromosomes, providing strong genetic evidence that this mutation in the human alpha

synuclein gene is causative for the PD phenotype in these families.

The Ala53Thr substitution is localized in a region of the protein whose secondary structure predicts an alpha helical formation, bounded by beta sheets. Substitution of the alanine with threonine is predicted to disrupt the alpha helix and extend the beta sheet structure. Beta pleated sheets are thought to be involved in the self aggregation of proteins which could lead to the formation of amyloid like structures (6).

This was already tested in the case of NAC35, the 35 amino acid peptide derived from alpha-synuclein that was first isolated from plaques found in patients with Alzheimer's disease (4). NAC35 was shown to self aggregate and form amyloid fibril which shared the 'amyloid' characteristics of insolubility in aqueous solutions and green birefringence under polarized light, subsequent to Congo red staining (6). NAC35 is located in the middle of the alpha synuclein molecule and extends from amino acid 61 to amino acid 95. Residue 53, which is found to be mutated in PD, is outside the NAC35 peptide found in amyloid plaques. However, the true size of the NAC peptide involved in the plaques is not known since the protease used to isolate the peptide from AD tissue cuts at lysine 60 of the alpha synuclein protein. It is therefore possible that amino acid 53 may be part of the NAC peptide found in plaques. In

crosslinking experiments with beta amyloid (Abeta), it was demonstrated (6) that residues 1-56 and 57-97 specifically bind amyloid and that a synthetic peptide consisting of residues 32-57 performed similarly.

Three members of the synuclein family have been characterized in the rat, with SYN1 exhibiting 95% homology with the human alpha-synuclein protein (7). SYN 1 of the rat is expressed in many regions of the brain, with high levels found in the olfactory bulb and tract, the hippocampus, dentate gyrus, habenula, amygdala and piriform cortex, and with intermediate levels in the granular layer of the cerebellum, substantia nigra, caudate-putamen, and dorsal raphe (7). This pattern of expression coincides with the distribution of the Lewy bodies found in brains of patients with Parkinson's disease. It is also interesting to note that decrease in olfactory sense often accompanies the syndromic features of Parkinson's disease, and in many cases it is proposed that hyposmia is a prodromic sign of the illness (8).

In the zebra-finch the homologue to alpha synuclein, synelfin, is thought to be involved in the process of song learning, suggesting a role for synuclein perhaps in memory and learning (9). In contrast to humans, rats have a threonine at residue 53 of their homologues to the human alpha synuclein gene (Figure 4). Similarly, the zebra-finch synelfin carries a threonine at amino acid 53, whereas both

Bos taurus and Torpedo californica do not (10). There are no reports that suggest the presence of Lewy bodies in the brains of the rat or the zebra finch or a phenotype resembling that of PD. Lack of any phenotype could be explained by a combination of factors, including the following: the relative short life span of rodents may prohibit the observation of a late onset disorder, interaction with other cellular components not present in the rat may be required for the phenotype, absence of a critical environmental trigger in the rodents, or finally a heterozygous status Ala/Thr may be necessary for the production of a phenotype.

Studies of early onset AD have previously documented that missense mutations can cause an adult onset neurodegenerative disorder. Of the 31 mutations described so far in the loci for presenilin 1 and 2, thirty were missense and one was a splice variant (11). Missense mutations in the prion protein have also been implicated in the amyloid production seen in Gerstmann-Straüssler-Scheinker and Creutzfeld-Jakob diseases, both forms of spongiform encephalopathy (12). Studies in these neurodegenerative disorders have pointed to the importance of the physical chemical properties of mutant cellular proteins in initiating and propagating neuronal lesions leading to disease. Similar studies in the synuclein protein family may provide valuable insights into the etiology and pathogenesis of PD.

Similarly with the mutations in the presenilin genes in patients with early onset Alzheimer's disease, the mutation identified in the alpha synuclein gene is unlikely to account for the majority of sporadic and familial cases of PD. However, this mutation may account for a significant proportion of those families with a highly penetrant, early onset autosomal dominant PD phenotype.

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

3. Summary of the Invention

As described herein, we have discovered that particular mutations in the alpha synuclein gene are associated with predisposition to Parkinson's disease. Accordingly, the present invention includes an isolated nucleic acid comprising a mutated synuclein gene. In particular, the isolated nucleic acid of the present invention contains at least one mutation in the alpha synuclein gene at base pair position 209 of Genbank # L08850, which, in particular, is a change from guanine to adenine. However, since other mutations in the alpha synuclein gene may also lead to Parkinson's Disease (PD), other mutations are also included. In addition, it is conceivable that mutations in the related beta (46)(SEQ ID NO 11) and gamma (SEQ ID NOs 12 and 13)

synuclein genes may also lead to PD. Thus, mutated homologues of the alpha synuclein gene are also included in the present invention. Vectors comprising the isolated nucleic acid and host cells comprising such vectors are included as well.

Knowledge of particular genes that are associated with PD allows for the search for other specific PD mutations. Accordingly, the present invention also includes a method of using a synuclein gene sequence to identify specific PD mutations. Such mutations may occur in an unrelated population or in a family that demonstrates passage of PD within the family tree.

Since knowledge of mutations associated with Parkinson's disease allows the development of genetic screens that test for an individual's chances of being predisposed to the disease, and such tests may be performed by hybridization analysis using oligonucleotides complementary to the sequence of interest or by PCR amplification using oligonucleotides that are complementary to sequences flanking the mutation, the present invention also includes oligonucleotides complementary to a portion of the synuclein gene, wherein said portion comprises or flanks a mutation associated with predisposition to Parkinson's Disease. In particular, the oligonucleotides of the present invention will have a sequence that is complementary to a sequence from the alpha synuclein gene that includes or flanks base pair position 209. And in particular, this mutation is a change from guanine to adenine at this position.

Vectors comprising an isolated nucleic acid encoding a mutated synuclein gene will allow the production and isolation of the mutant protein in an appropriate host cell using techniques well known in the art. Alternatively, peptides may be chemically synthesized using techniques also well known in the art. Isolation of such a protein or peptides thereof will allow the study of the molecular mechanisms which lead to development of Parkinson's disease. Accordingly, the present invention also includes an isolated synuclein protein or peptide containing at least one mutation. In particular, this mutation is at a position corresponding to the fifty-third amino acid in the native alpha synuclein protein, and in particular, this mutation is an alanine to threonine substitution.

Peptides corresponding to portions or the entirety of a synuclein gene may be useful as drugs for inhibiting the self-aggregation of mutant proteins that is thought to lead to Parkinson's disease. Accordingly, the present invention includes a method of testing peptides and other compounds for the ability to interfere with this self-aggregation. Self-aggregation can be tested using a number of established methods, including Congo red staining, electron microscopy pictures of amyloid fibrils, and circular dichroism (CD) spectrophotometry. Using a peptide derived from the alpha synuclein protein that includes the mutant THR amino acid at position 53 alone or in combination with a normal peptide may allow testing for drugs that can inhibit the aggregation or dissolve an aggregate. This procedure can be used to rapidly

identify agents that could be used in animal studies, clinical trials, or as diagnostic tools.

Possession of isolated synuclein proteins or peptides will also allow the isolation of specific antibodies using techniques well known in the art. Such antibodies may distinguish a mutant synuclein protein from its wildtype counterpart, and therefor could also be used in diagnostic screens. Alternatively, such antibodies may also be used to inhibit the self-aggregation of proteins during the progression of Parkinson's disease. Accordingly, the present invention also includes antibodies specific for a mutated synuclein protein or peptide. It should be understood that useful derivatives of such antibodies, such as Fv fragments and Fab fragments, are also included.

The above aspects of the present invention will allow methods of detecting subjects at increased risk for Parkinson's Disease. Such a method comprises obtaining a sample comprising nucleic acids from the subjects, and detecting in the nucleic acids the presence of a mutation which is associated with Parkinson's disease. In particular, the mutation detected by the method of the present invention is located on human chromosome four, preferably in the alpha synuclein gene. In particular, the mutation causes an amino acid substitution at position 53 of the alpha synuclein gene, which is, in particular, an alanine to threonine substitution.

The detecting step of the method of the present invention may be accomplished several different ways as will

be described in further depth below. All such methods are well known to those of ordinary skill in the art.

For instance, said detecting step may comprise combining a nucleotide probe which selectively hybridizes to a nucleic acid containing a mutation associated with a predisposition to Parkinson's disease, and detecting the presence of hybridization. Such a probe may be an oligonucleotide that is complementary to a portion of the synuclein gene, wherein said portion comprises the mutation. In particular, such an oligonucleotide is complementary to a mutated alpha synuclein gene having at least one mutation at base pair position 209. In particular, this mutation is a change from guanine to adenine.

The detecting step of the method of the present invention may also comprise amplifying a nucleic acid product comprising said mutation, and detecting the presence of said mutation in the amplified product using any nucleic acid sequencing procedure known in the art. Alternatively, the detecting step may comprise selectively amplifying a nucleic acid product comprising said mutation, and detecting the presence of amplification using any appropriate method known in the art. Such methods include gel electrophoresis of amplified nucleic acids, and detection of radiolabeled amplified nucleic acids using autoradiographic film or any other detection method known in the art.

The amplifying step of the present invention may be performed using the polymerase chain reaction (PCR), reverse transcriptase PCR (RTPCR), or any other type of PCR reaction

known in the art. Accordingly, such a step will comprise at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids. In particular, said amplifying step uses two oligonucleotides. And in particular, the two oligonucleotides have the sequences given in SEQ ID NOs 2 and 3.

Alternatively, the detecting step of the method of the present invention comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of a nucleic acid sample. Such a detecting means will be possible when a mutation associated with a predisposition to Parkinson's disease results in a sequence having a new restriction endonuclease cleavage site, or loss of a native restriction endonuclease site. In particular, the mutation associated with Parkinson's disease results in the formation of a non-native *Tsp*45I restriction endonuclease site.

Alternatively, the detecting step of the present invention may be performed using a gene-specific primer and subsequent chain termination at the position of the mutation using DNA polymerase and labeled nucleotides or dideoxynucleotides. The presence of nucleic acids in which a dideoxynucleotide corresponding to the mutation of interest is incorporated at the appropriate position may be detected by any means known in the art, including detection of radiolabeled dideoxynucleotides using, for example, autoradiographic film, or detection of fluorescently-labeled dideoxynucleotides.

Since the methods and compounds of the present invention

will be useful in diagnostic screening procedures aimed at identifying individuals having a predisposition for Parkinson's disease, the present invention also includes diagnostic kits which include the compounds of the present invention in a form that allows such compounds to be used quickly and easily for the designated purpose.

Finally, the inventors also contemplate that the isolated nucleic acid, oligonucleotides and antibodies of the present invention may eventually be used in methods directed at the correction or suppression of Parkinson's disease. For example, oligonucleotides or expression vectors designed from the synuclein nucleic acid sequences of the present invention may one day be used in antisense therapy directed at inhibiting expression of the mutated synuclein protein in patients with Parkinson's disease, or in individuals having a predisposition for Parkinson's disease. Similarly, antibodies specific for the mutated synuclein protein may be useful in therapies directed at inhibiting the self-aggregation of mutated proteins or peptides in patients having Parkinson's disease. Knowledge of gene(s) associated with the development of Parkinson's disease may also allow the design of transgenic animals which express the mutant gene(s). Such animals may serve as a useful disease model, allowing one to test the effects of candidate therapies and therapeutic compositions in the treatment or inhibition of Parkinson's disease.

A detailed description of the present invention is now provided, and should not be considered as limiting on the present invention as described above.

4. Brief Description of the Drawings

Figure 1.

DNA sequence of the PCR product used for mutation detection (SEQ ID NO 1). Oligonuclectide primers are shown by arrows and the numerals 3 and 13 (SEQ ID NO 2 and 3). Intron sequence is shown in lower case and exon sequence in upper case. Amino acid translation of the exon is shown below the DNA sequence. The circled base represents the G209A change in the mutant allele. The resulting amino acid Ala53Thr change is represented by the circled amino acid. The newly created *Tsp*45 I site is indicated above the DNA sequence.

Figure 2.

Mutation analysis of the G209A change is shown in a subpedigree of the Italian kindred. Filled symbols represent affected individuals. Numerical identifiers, denote the individuals immediately above. *Tsp*45 I digestion of PCR products is shown at the bottom of the figure, and fragment sizes are indicated on the right in base pairs.

Figure 3.

Mutation analysis of the G209A change in RT PCR products (7). Lane 1: 100 bp ladder, lanes 2 and 3 normal control, lanes 4 and 5 PD patient, lane 6 negative control without RT enzyme. Sizes are indicated on the right in base pairs. Lanes 2 and 4 show uncut DNA and lanes 3 and 5 show DNA cut with *Tsp*45 I.

Figure 4.

Sequence alignments of alpha synuclein homologues in different species. Accession numbers for the sequences used were as follows: Homo sapiens Swiss-Prot P37840 (SEQ ID NO 4), Rattus norvegicus Swiss-Prot P37377 (SEQ ID NO 5), Bos taurus Swiss-Prot P33567 (SEQ ID NO 6), Serinus canaria genbank L33860 (SEQ ID NO 7), Torpedo californica Swiss-Prot P37379 (SEQ ID NO 8). Numbering on top of the alignments is according to the human sequence. Amino acid 53, which is the site of the Ala53Thr change, is circled.

Figure 5.

The pedigree of a large family with PD (3). The clinical and pathological features of some members of this kindred were previously reported.

Figure 6.

Multipoint LOD score analysis between chromosome 4q markers and the PD locus.

Figure 7.

A table of human synuclein clones identified from various databases. Columns labeled 5' and 3' show the sequence acquisition numbers. Clones were identified by homology to protein or nucleic acid sequence. Human gamma clones were identified by homology to known mouse and rat gamma synuclein sequences.

Figure 8.

Sequence of BAC clone 139A20 for human beta synuclein. BAC clone was isolated using primers to known database sequences described in Figure 7. The sequence shown includes all coding exon sequences and some non-coding intronic sequences.(SEQ ID NO:11)

Figure 9.

Sequence from the 5' end of BAC clone 174P13 for human gamma synuclein. The BAC clone was isolated with primers from the database sequences described in Figure 7.(SEQ ID NO:12)

Figure 10.

Sequence from the 3' end of BAC clone 174P13 for human gamma synuclein. BAC clone was isolated as described in Figure 9. The entire human gamma synuclein gene has now been sequenced and has been deposited in GenBank: accession number

AF044311.(SEQ ID NO: 13)

Figure 11.

Sequence of exons 1-7 of the human alpha synuclein gene, plus some flanking intronic sequence for each exon.(SEQ ID NOs 14-19)

5. Detailed Description of the Invention

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are defined below.

This invention provides a method of diagnosing or predicting a predisposition to Parkinson's disease. The method comprises detecting in a sample from a subject the presence of a mutation, for example, in nucleotide position 209 of the human alpha synuclein gene. The presence of the mutation indicates the presence of or a predisposition to Parkinson's disease.

As used herein, the term "gene" primarily relates to a coding sequence, but can also include some or all of the surrounding or flanking regulatory regions or introns. The term "gene" specifically includes artificial or recombinant

genes created from cDNA or genomic DNA, including recombinant genes based upon splice variants.

As used herein, the term "synuclein" gene or protein may refer to the alpha synuclein gene or any homologue thereof. A "homologue" is understood to mean any related gene or protein that is at least 25% homologous to the alpha synuclein gene or protein or performs a related function. Preferably, a synuclein gene or protein refers to alpha, beta or gamma synuclein, but most preferably refers to alpha synuclein.

As used herein, an "isolated nucleic acid" is a ribonucleic acid, deoxyribonucleic acid, or nucleic acid analog comprising a polynucleotide sequence that has been isolated or separated from sequences that are immediately contiguous, i.e. on the 5' and 3' ends, in the naturally occurring genome of the organism from which it is derived. The term therefor includes, for example, a recombinant nucleic acid which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule independent from any other sequences.

An isolated nucleic acid of the present invention may be "operatively linked" to an expression control sequence or regulatory region. As used herein, "operatively linked" means that the components are joined in such a way that the expression, transcription or translation of the sequence is under the influence or control of the regulatory region.

As used herein, a "predisposition" to Parkinson's

disease means an increased probability of developing Parkinson's disease during the subject's lifetime as compared to the average individual.

Pertaining to this probability, a LOD score is a measure of genetic linkage used herein, defined as the log$_{10}$ ratio of the probability that the data would have arisen if the loci are linked to the probability that the data could have arisen from unlinked loci. The conventional threshold for declaring linkage is a LOD score of 3.0, that is, a 1000:1 ratio (which must be compared with the 50:1 probability that any random pair of loci will be unlinked).

As used herein, reference to "base pair position" or "amino acid position" when referring to an isolated nucleic acid, probe, protein or peptide always indicates the relative position in the native gene or protein.

A "probe" refers to a nucleic acid which has sufficient nucleotides surrounding the codons at the mutation positions to distinguish the nucleic acid from nucleic acids encoding non-related genes. The specific length of the nucleic acid is a matter of routine choice based on the desired function of the sequence. For example, if one is making probes to detect the mutation in base pair position 209, the length of the nucleic acid is preferably small, but must be long enough to prevent hybridization to undesired background sequences. However, if the desired hybridization is to a nucleic acid which has been amplified, background hybridization is less of a concern and a smaller probe can be used. In general, such a probe will be between 10 and 100 nucleotides, especially

between 10 and 40 and preferably between 15 and 25 nucleotides in length. It is apparent to one of skill in the art that nucleotide substitutions, deletions, and additions may be incorporated into the polynucleotides of the invention. However, such nucleotide substitutions, deletions, and additions should not substantially disrupt the ability of the polynucleotide to hybridize under conditions that are sufficiently stringent to result in specific hybridization.

As used herein with respect to genes, "the term "normal" refers to a gene which encodes a normal protein. As used herein with respect to proteins, the term "normal" means a protein which performs its usual or normal physiological role and which is not associated with, or causative of, a pathogenic condition or state. Therefor, the term "normal" is generally synonomous with the phrase "wild type".

For any given gene or corresponding protein, a multiplicity of normal allelic variants may exist, none of which is associated with the development of a pathogenic condition or disease state. Such normal allelic variants include, but are not limited to, variants in which one or more nucleotide substitutions do not result in a change in the encoded amino acid sequence.

As used herein, the term "mutation" generally refers to a mutation in a gene that is associated with a predisposition to Parkinson's disease. "Mutant" can specifically refer to a mutation at nucleotide position 209 of the synuclein gene, and is in particularly a G to A transition. However, other mutations in the synuclein gene or other genes which are

associated with a predisposition to Parkinson's disease are also encompassed. Furthermore, the term "mutation" is not limited to transition mutations, but can also mean a deletion, insertion or transversion as well.

The term "mutant", as it applies to synuclein genes, is not intended to embrace sequence variants which, due to the degeneracy of the genetic code, encode proteins identical to the normal sequences disclosed or otherwise enabled herein; nor is it intended to embrace sequence variants which, although they encode different proteins, encode proteins which are functionally equivalent to normal synuclein proteins. The term "mutant" means a protein which does not perform its usual or normal physiological role and which is associated with, or causative of, a pathogenic condition or state.

Since a mutation can be a substitution, deletion or insertion, a mutated synuclein "protein" is understood to refer to the amino acid sequence resulting from any such mutation whether the resulting protein is shorter, longer or modified, i.e. due to an alteration in reading frame or generation of stop codon. In addition, "peptide" is understood to refer to a portion of the mutated protein that is preferably at least five base pairs long, and more preferably at least 10 base pairs long. This portion may be derived from the amino or carboxyl terminus, or it may be an internal portion of the full length protein. As such, a peptide may be chemically synthesized using any method known in the art, or may be made using a recombinant DNA technology

and an appropriate purification scheme or isolated from the native protein using enzymatic digestion.

As used herein, the term "substantially pure" means a preparation which is at least 60% by weight the compound of interest. Preferably the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate method, i.e. column chromotography, gel electrophoresis or HPLC analysis.

"Specific or selective hybridization" as used herein means the formation of hybrids between a probe nucleic acid (e.g., a nucleic acid which may include substitutions, deletions, and/or additions) and a specific target nucleic acid (e.g., a nucleic acid having the mutated sequence), wherein the probe preferentially hybridizes to the specific target such that, for example, a band corresponding to the mutated DNA or restriction fragment thereof can be identified on a Southern blot, whereas a corresponding normal or wild-type DNA is not identified or can be discriminated from a variant DNA on the basis of signal intensity. Hybridization probes capable of specific hybridization to detect a single-base mismatch may be designed according to methods known in the art (13-17).

"Stringent" as it refers to hybridization conditions is a term of art understood by those of ordinary skill to refer to those conditions of temperature, chaotrophic acids, buffer and ionic strength which permit hybridization of a particular nucleic acid sequence to its complementary sequence and not

to substantially different sequences. The exact conditions which constitute "stringent" conditions depend on the nature of the nucleic acid sequence, the length of the sequence, and the frequency of occurrence of subsets of that sequence within other non-identical sequences. By varying hybridization conditions from a level of stringency at which non-specific hybridization occurs to a level at which only specific hybridization occurs, one of ordinary skill in the art can, without undue experimentation, determine conditions which will allow a given sequence to hybridize only with complementary sequences.

Suitable ranges of stringency conditions are described in Sambrook et al. (13). Hybridization conditions, depending on the length and commonality of a sequence, may include temperatures of 20°C-65°C and ionic strengths from 5X to 0.1X SSC. Highly stringent hybridization conditions may include temperatures as low as 40°C-42°C (when denaturants such as formamide are included) or up to 60°C-65°C in ionic strengths as low as 0.1X SSC. These ranges are, however, only illustrative and, depending on the nature of the target sequence, and possible future technological developments, may be more stringent than necessary. Appropriate conditions may be determined for each specific nucleic acid sequence or oligonucleotide probe using standard control and a level of experimentation that is not considered to be undue by those of skill in the art.

As discussed below in greater detail, the mutation can be detected by many methods. For example, the detecting step can comprise combining a nucleotide probe capable of selectively hybridizing to a nucleic acid containing the mutation with a nucleic acid in the sample and detecting the presence of hybridization. Additionally, the detecting step can comprise amplifying the nucleotides surrounding and including the mutation and detecting the presence of the mutation in the amplified product, or selectively amplifying the nucleotides of the mutation and detecting the presence of amplification. Finally, the detecting step can comprise detecting the presence or absence of a restriction fragment created by an enzyme digest of the sample nucleic acid, or any other detection means known in the art.

Detection Techniques

Once the location of a PD-relevant mutation is known, the methods to detect such a mutation are standard in the art. The sequence of various nucleotide probes can be determined from the known sequence of the relevant gene, especially the sequences surrounding the mutation.

Detection of point mutations using direct probing involves the use of oligonucleotide probes which may be prepared, for example, synthetically or by nick translation. The probes may be suitably labeled using, for example, a radio label, enzyme label, fluorescent label, biotin-avidin label and the like for subsequent visualization by any appropriate assay, i.e. Southern blot hybridization. In this procedure, the labeled

probe is reacted with sample DNA that is bound, for example, to a nylon filter under conditions such that only fully complementary sequences hybridize. The areas that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling may then be visualized, for example, by autoradiography.

Methods of manipulating hybridization conditions to achieve varying degrees of specificity are well known in the art. For example, tetra-alkyl ammonium salts may be used to bind selectively to A-T base pairs, thus displacing the dissociation equilibrium and raising the melting temperature. At 3M Me 4NCl, this is sufficient to shift the melting temperature to that of G-C pairs. This results in a marked sharpening of the melting profile. The stringency of hybridization in such an experiment is usually 5°C below the Ti (the irreversible melting temperature of the hybrid formed between the probe and its target sequence) for the given chain length. For a 20mer oligonucleotide probe, the recommended hybridization temperature is about 58°C. The washing temperatures are unique to the sequence under investigation and need to be optimized for each variant.

There are certainly other ways known in the art for adjusting hybridization conditions in view of desired specificity. For instance, although hybridization may be carried out in accordance with conventional hybridization methods under suitable conditions with respect to e.g. stringency, incubation time, temperature, etc, the choice of

conditions will depend on the desired degree of complementarity between the fragments to be hybridized. A high degree of complementarity requires more stringent conditions such as low salt concentrations, low ionic strength of the buffer and higher temperatures, whereas a low degree of complementarity requires less stringent conditions, e.g. higher salt concentration, higher ionic strength of the buffer or lower temperatures, for the hybridization to take place.

The support to which DNA or RNA fragments of the sample to be analyzed are bound in denatured form is preferably a solid support and may have any convenient shape. Thus, it may, for instance, be in the form of a plate, e.g. a thin layer or a microtiter plate, a strip, a solid particle e.g. in the form of a bead such as a latex bead, a filter, a film or paper. The solid support may be composed of a polymer, preferably nylon or nitrocellulose.

Alternative probing techniques, such as ligase chain reaction (LCR), may involve the use of mismatch probes, i.e., probes which are fully complementary with the target except at the point of the mutation. The target sequence is then allowed to hybridize both with oligonucleotides which are fully complementary and have oligonucleotides containing a mismatch, under conditions which will distinguish between the two. By manipulating the reaction conditions according to the above considerations, it is possible to obtain hybridization only where there is full complementarity. If a mismatch is present there is significantly reduced hybridization.

The polymerase chain reaction (PCR) is a technique that amplifies specific DNA sequences with remarkable efficiency. Repeated cycles of denaturation, primer annealing and extension carried out with Taq polymerase, e.g., a heat stable DNA polymerase, leads to exponential increases in the concentration of desired DNA sequences. Given a knowledge of the nucleotide sequence of the mutations, synthetic oligonucleotides can be prepared which are complementary to sequences which flank the DNA of interest. Each oligonucleotide is complementary to one of the two strands. The DNA is denatured at high temperatures (e.g., 95°C) and then reannealed in the presence of a large molar excess of oligonucleotides. The oligonucleotides, oriented with their 3' ends pointing towards each other, hybridize to opposite strands of the target sequence and prime enzymatic extension along the nucleic acid template in the presence of the four deoxyribonucleotide triphosphates. The end product is then denatured again for another cycle. After this three-step cycle has been repeated several times, amplification of a DNA segment by more than one million-fold can be achieved. The resulting DNA may then be directly sequenced in order to locate any genetic alteration. Alternatively, it may be possible to prepare oligonucleotides that will only bind to altered DNA, so that PCR will only result in multiplication of the DNA if the mutation is present. Following PCR, direct visualization or allele-specific oligonucleotide hybridization (18) may be used to detect the Parkinson's disease point mutation. Alternatively, PCR may be followed

by restriction endonuclease digestion with subsequent analysis of the resultant products.

As shown in the examples, the substitution of G for A at base pair 209 of the synuclein, results in the gain of a *Tsp*45I site. The gain of this restriction endonuclease recognition site facilitates the detection of the Parkinson's disease mutation using restriction fragment length polymorphism (RFLP) analysis or by detection of the presence or absence of the restriction site in a PCR product that spans base pair position 209.

For RFLP analysis, DNA is obtained, for example from the blood cells of the subject suspected of having Parkinson's disease and from a normal subject, is digested with a restriction endonuclease, and subsequently separated on the basis of size by agarose gel electrophoresis. The Southern technique can then be used to detect, by hybridization with labeled probes, the products of endonuclease digestion. The patterns obtained from the Southern blot can then be compared. Using such an approach, an additional restriction endonuclease site, such as a *Tsp*45I site, is detected by determining the number of bands detected and comparing this number to the normal subject.

The creation of a new restriction site as a result of a nucleotide substitution at a disclosed mutation site can be readily determined by reference to the genetic code and a list of nucleotide sequences recognized by restriction endonucleases (19).

In general, primers for PCR are usually about 20 bp in

length, and are most preferably 15-25 bp. Denaturation of strands usually takes place at 94°C. and extension from the primers is usually at 72°C. The annealing temperature varies according to the sequence under investigation. Examples of reaction times are: 20 mins denaturing; 35 cycles of 2 min, 1 min, 1 min for annealing, extension and denaturation; and finally a 5 min extension step.

PCR "amplification of specific alleles" (PASA) may also be used to detect the presence of the PD mutation. PASA is a rapid method of detecting single-base mutations or polymorphisms (22-28). PASA (also known as allele specific amplification) involves amplification with two oligonucleotide primers such that one is allele-specific. The desired allele is efficiently amplified, while the other allele(s) is poorly amplified because it mismatches with a base at or near the 3' end of the allele-specific primer. Thus, PASA or the related method of PAMSA may be used to specifically amplify the mutation sequences of the invention. Where such amplification is done on genetic material (or RNA) obtained from an individual, it can serve as a method of detecting the presence of the mutations.

As mentioned above, a method known as ligase chain reaction (LCR) can be used to successfully detect a single-base substitution (29, 30). LCR probes may be combined or multiplexed for simultaneously screening for multiple different mutations. Thus, LCR can be particularly useful where multiple mutations are predictive of the same disease.

Finally, the Parkinson's disease mutation of the present invention may also be detected using chain termination with labeled dideoxynucleotides. For instance, U.S. Patent No. 5,047,519 to Hobbs et al. discloses fluorescently-labeled nucleotides as chain-terminating substrates for a fluorescence-based DNA sequencing method. With such substrates and knowledge of the gene sequence of interest, it is possible to design an assay using a gene-specific primer to initiate a polymerase reaction immediately flanking the position of the mutation, employing color-coded dideoxynucleotide terminators such that the specific nucleotide at the position of the mutation may be easily determined via a colorimetric assay.

Transgenic Animals and Cell Lines

Having identified subjects having a predisposition to Parkinson's disease associated with a specific mutation, the subjects can participate in the screening of putative agents capable of treating Parkinson's disease. This method comprises administering the test agent to the subject, which may be a human, which has a mutation in a gene associated with Parkinson's disease and monitoring the effect of the agent on the subject's condition. If the symptoms of Parkinson's disease improve, the agent can be used as a treatment for the disease.

In addition, it is possible to develop transgenic model systems and/or cell lines containing the mutated nucleic acid(s) for use, for example, as model systems for screening

for drugs and evaluating drug efficiency. Additionally, such model systems provide a tool for defining the underlying biochemistry of, for instance, the mutated synuclein gene, thereby providing a rationale for drug design.

One approach to creating transgenic animals is to mutate the animal gene of interest by in vivo mutagenesis, transfer the mutant gene into embryonic stem cells by DNA transfection and inject the embryonic stem cells into blastocysts in order to retrieve offspring which carry the disease-causing mutation (31). Alternatively, the technique of microinjection of the mutated gene, into a one-cell embryo followed by incubation in a foster mother can be used. Alternatively, viral vectors, e.g., Adeno-associated virus, can be used to deliver the mutated gene to a stem cell, or may be used to target specific cells of a fully developed animal (32,33).

Antibodies and Recombinant Expression of Polypeptides

When the mutated gene product is a polypeptide, e.g. the 209 mutation, it can be used to prepare antisera and monoclonal antibodies using, for example, the method of Kohler and Milstein (34). Such monoclonal antibodies could then form the basis of a diagnostic test, or may even be useful in therapies directed toward inhibiting the action of the mutant protein in a patient with Parkinson's disease.

Mutant polypeptides can also be used to immunize an animal for the production of polyclonal antiserum (35). For example, a recombinantly produced fragment of a variant polypeptide can be injected into a mouse along with an

adjuvant so as to generate an immune response. Murine immunoglobulins which specifically bind the recombinant fragment can be harvested from the immunized mouse as an antiserum, and may be further purified by affinity chromatography or other means. Additionally, spleen cells are harvested from the mouse and fused to myeloma cells to produce a bank of antibody-secreting hybridoma cells, which can then be screened for clones that secrete immunoglobulins which bind the recombinantly produced fragment with an increased affinity. More specifically, immunoglobulins that selectively bind to the variant polypeptides but poorly or not at all to wild-type polypeptides are selected, either by pre-absorption with wild-type proteins or by screening of hybridoma cell lines for specific idiotypes that bind the variant but not wild-type.

These antibodies can be used to screen protein and tissue samples for the presence of mutated proteins. A colored enzymatic reaction occurs when the specific antibody remains bound to its target protein, in situ, after thorough washing, as directed by established protocols.

Gene expression

The nucleic acid sequences of the present invention will be capable of expressing the desired mutant or normal polypeptides in an appropriate host cell. For expression in host cells, the DNA sequences of the present invention will be operably linked to, i.e., positioned to ensure the functioning of, an expression control sequence. For example,

such polynucleotides can include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site, and, optionally, an enhancer for use in eukaryotic expression hosts. In addition, the DNA sequence of the present invention may also be fused such that the reading frame is conserved to an appropriate signal sequence to facilitate export of the encoded protein across the cell membrane.

Expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. A variety of suitable expression vectors are disclosed in Sambrook et al. (13). Commonly, expression vectors will contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired DNA sequences.

E. coli is one prokaryotic host that is particularly useful for cloning and expression of the DNA sequences of the present invention because of the wide variety of available expression systems. Vectors suitable for use in E. coli are known and are commercially available, i.e. pBR322 (13), pBLUESCRIPT (Stratagene), etc. Also, a variety of different types of expression systems may be used, including plasmids, cosmids, bacteriophage lambda, etc. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilus, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. Expression vectors for use in prokaryotic host cells will typically contain expression

control sequences compatible with the host cell (e.g., an origin of replication). In addition, any of a variety of well-known promoters may be used, such as the lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. A promoter may optionally contain an operator sequence for regulatable gene expression, and will have a ribosome binding site sequence for the initiation of translation.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (36). Vectors for use in eukaryotic cells are known and commercially available, i.e. pcDNA3 (Invitrogen). Eukaryotic cells are actually preferred, and a number of suitable host cell lines capable of secreting intact human proteins have been developed in the art, including CHO cells, COS cells, HeLa cells, myeloma cell lines, Jurkat cells, etc. Promoters for use in eukaryotic vectors may be cell-specific, or capable of being expressed in a wide variety of cells, i.e. viral promoters.

Expression vectors of the present invention (e.g., comprising nucleic acid sequences encoding a mutant or normal polypeptide) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts.

Kits

The method lends itself readily to the formulation of test kits which can be utilized in diagnosis. Such a kit would comprise a carrier compartmentalized to receive in close confinement one or more containers wherein a first container may contain suitably labeled DNA probes. Other containers may contain reagents useful in the localization of the labeled probes, such as enzyme substrates. Still other containers may contain restriction enzymes (such as *Tsp*45I), buffers, etc., together with instructions for use.

DESCRIPTION OF THE INVENTION

Detailed Description of the Preferred Embodiments

The following laboratory procedures were used:

DNA samples were collected upon informed consent. High molecular weight genomic DNA was isolated from whole-blood lysate by methods previously described (38). Genotyping was performed as previously described (39). Pairwise linkage analysis was performed using the MLINK program of the FASTLINK package (40-42). Allele frequencies were used as reported in the Genomic Data Base (http://gdbwww.gdb.org) and the Cooperative Human Linkage Consortium (CHLC) database (http://www.chlc.org). Multipoint analysis was performed using the LINKMAP program of the FASTLINK package. For the multipoint analysis allele frequencies were set to 1/n where n equals the number of alleles observed. In the two point analysis LOD scores were calculated for both the reported and the 1/n allele frequencies with minimal effect on the

maximum LOD score observed. Simulations of multipoint analysis in a subset of the pedigree with different allele frequencies similarly indicated no significant effect on the scores calculated. Maximum LOD scores as shown were observed for the heterozygote and homozygote disease allele penetrance set to 0.99, which is similar to the PD allele penetrance previously reported ranging from 0.88 to 0.94 (3). All unaffected individuals used in the study were of age above the mean for onset of illness. Disease allele frequency was set to 0.0001. Distances and order of genetic markers were set as reported in the CHLC database. Overlapping three point analysis was performed for markers *D4S2361, D4S1647, D4S421* and the PD locus. The 12 allele *D4S2380* locus was not included because of prohibitive time run. Multipoint analysis was performed on an IBM SP2 parallel computer and the SGI Challenge machine.

For mutation analysis genomic DNA was amplified with oligonucleotides (3): 5' GCTAATCAGCAATTTAAGGCTAG 3' (SEQ ID NO 2) and (13): 5' GATATGTTCTTAGATGCTCAG 3' (SEQ ID NO 3) of genbank ID: U46898, under standard PCR conditions. Sequence analysis was performed using the Perkin Elmer dye terminator cycle sequencing kit on an ABI 373 fluorescent sequencer (ABI, Foster City, CA). Restriction digestion was performed following the PCR with *Tsp*45 I according to manufacturer's protocol (New England Biolabs, Beverly, MA). The digested PCR products were electrophoresed on a 6% Visigel (Stratagene, La Jolla, CA), and visualized by ethidium

bromide staining. Pedigree structure in Figure 2 has been slightly modified in order to protect patient confidentiality.

Total RNA was extracted from the lymphoblastoid cell line of an affected individual and first strand synthesis was performed by oligo dT priming (Gibco BRL, Gaithersburg, MD). Primers (1F) 5' ACGACAGTGTGGTGTAAAGG 3' (SEQ ID NO 9) and (13R) 5' AACATCTGTCAGCAGATCTC 3' (SEQ ID NO 10) corresponding to nucleotides 21-40 and 520-501 of genbank L08850 were used to amplify a product of 500 bp containing the mutation at nucleotide 209. PCR products were subjected to restriction digestion by *Tsp*45 I. The mutation at nt 209 creates a novel *Tsp*45 I site (Figure 1), so that the normal allele will be restricted in 4 fragments of 249, 218, 24 and 9 bp, where the mutant allele will have 5 fragments of 249, 185, 33, 24 and 9 bp of size, as shown in Figure 3. Size standards used, were the 100 bp ladder (Gibco BRL, Gaithersburg, MD).

Example 1

In an effort to identify a genetic locus responsible for Parkinson's disease, we performed a genome scan in a large kindred of Italian descent with pathologically confirmed PD (Figure 5). The kindred originated in the town of Contursi in the Salerno province of Southern Italy (3). Some members emigrated to the United States, Germany and other countries. The extended family pedigree consists of 592 members with 60 individuals affected by PD. The average age of onset for the

illness in this pedigree (Figure 5) has been shown to be 46 ± 13 years. One hundred and fourty genetic markers were typed in this pedigree at an average spacing of about 20 cm. Genetic markers at the cytogenetic location *4q21-q23* were the only ones to show linkage to the disease phenotype with a Zmax=6.00 at theta=0.00 for marker *D4S2380I* (see Table 1).

Table 1. Two point LOD scores between chromosome 4q markers and the PD locus

	Two-point LOD scores at recombination fractions of:								
Locus	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Z_{max}	θ_{max}
D4S2361	-5.60	-0.83	0.30	0.54	0.43	0.21	0.06	0.55	0.12
D4S2380	6.00	5.90	5.30	4.60	3.00	1.50	0.50	6.00	0.00
D4S1647	5.22	5.07	4.47	3.71	2.26	1.05	0.30	5.22	0.00
D4S421	-2.42	0.45	0.77	0.65	0.38	0.22	0.09	0.77	0.05

Recombinations between the disease phenotype and genetic markers were observed in the proximal region for marker *D4S2361* and in the distal region for marker *D4S421*. Genetic markers *D4S2380* and *D4S1647* showed no obligate recombination events in the affected individuals.

Multipoint LOD score analysis between markers *D4S2361*-13cM-*D4S1647*-3cM-*D4S421* and the disease locus places the PD gene between markers *D4S2361* and *D4S421* at a recombination distance of 0.00 cM from marker *D4S1647* with a Zmax=6.04 (Figure 6). This location is favored from the alternative genetic intervals by a difference in the LOD score of greater

than three LOD units.

Although expansions of unstable trinucleotide repeats are found in a number of human neurogenerative conditions, there is no evidence for an association of a CAG trinucleotide repeat expansion in families with PD (43). In addition, genetic linkage studies in other families with PD-like illnesses do not support the involvement of several candidate genes (glutathione peroxidase, tyrosine hydroxylase, brain-derived neurotrophic factor, catalase, amyloid precursor protein, CuZn superoxide dismutase and debrisoquinone 4-hydroxylase) in the etiology of the disorder (44). Genes previously mapped in the general region of linkage include the loci for alcohol dehydrogenase, formaldehyde dehydrogenase, synuclein, UDP-N-acetylglycosamine phosphotransferase and others.

Our localization of a PD susceptibility gene represents the first genetic locus linked to PD. Other distinct clinicopathological entities associated with parksonian features are probably linked to other genetic loci. For example, Wilhelmsen-Lynch disease (disinhibition-dementia-parkinsonian-amyotrophy complex) is linked to the *17q21-q22* chromosomal region (45). If the pathogenesis of diseases affecting the nigrostriatal pathway includes environmental influences, then a range of mutations affecting vulnerable sites in the electron transport chain or enzyme polymorphisms influencing neurotoxin metabolism may vary the penetrance of PD by altering an individual's resistance to exogenous or endogenous agents. However, our finding of a highly

penetrant genetic locus linked to PD suggested that abnormalities of a single gene may be sufficient to cause Parkinson's disease.

Example 2

In an effort to identify a specific gene between markers *D4S2361* and *D4S421* that is associated with predisposition to Parkinson's disease, we conducted sequence analysis of candidate genes in this region.

Alpha synuclein, a presynaptic nerve terminal protein, was originally identified as the precursor protein for the NAC peptide, a non beta amyloid component of Alzheimer's disease (AD) amyloid plaques (*4*). The human alpha synuclein gene was previously mapped in the 4q21-q22 region (*5*). We refined the mapping, and determined that the alpha synuclein gene is located within the non-excluded region harboring the PD gene in the Italian kindred. Thus alpha synuclein represented an excellent candidate locus for PD.

Sequence analysis of the fourth exon of the alpha synuclein gene revealed a single base pair G209A change from the published sequence of the gene (GenBank ID L08850), which results in an Ala53Thr substitution and the creation of a novel *Tsp*45 I restriction site (Figure 1). Mutation analysis for the G209A change in the Italian kindred shows complete segregation with the PD phenotype with exception of

individual 30 (Figure 2), who is affected but not carrying this mutation. This individual apparently inherited a different PD mutation from his father, as we have shown that he shares a genetic haplotype with his unaffected maternal uncle, individual 3, for genetic markers in the PD linkage region.

The frequency of this variation was studied in two general population samples, one consisting of 120 chromosomes of the parents of the CEPH reference families, and the other consisting of 194 chromosomes of unrelated individuals from the blood bank in Salerno, Italy, a city near the town from which the family originated. Of these 314 general population chromosomes none was found to carry the G209A mutation. Fifty two patients of Italian descent with sporadic PD were also screened for the mutation (Figure 2), along with 5 probands from previously unpublished Greek families with PD. The Ala53Thr change was found to be present in three of the Greek kindreds and it segregated with the PD phenotype. In those three Greek kindreds it is worth noting that the age of onset for the disease is relatively early, ranging from the mid 30's to the mid 50's. Extended haplotype analysis of the Greek kindreds and the Italian PD family suggests that the mutations arose independently on different ancestral chromosomes. The finding of the Ala53Thr substitution in four independent PD families and its absence from 314 control chromosomes provides the strongest genetic evidence that this

mutation in the human alpha synuclein gene is causative for the PD phenotype in these families.

We have also demonstrated by RT PCR that the mutant allele is transcribed in the lymphoblast cell line of an affected individual from the Italian kindred (Figure 3) (7). Thus, it is reasonable to assume that the mutant protein is indeed expressed.

Example 3.

Since homologous genes that are related to the alpha synuclein protein have been identified in other species, it seemed reasonable to assume that homologues of alpha synuclein would exist in humans as well. In fact, human beta synuclein has previously been described (46), and is approximately 60% similar to alpha synuclein at the protein level.

We set out to identify other related homologues by searching various databases for homologous genes and proteins. Protein sequence databases searched included the NR (non-redundant) and "month" databases of Genbank and Swiss Prot. Nucleotide databases included NR, month, dbstf, GSS (Genome Sequence Service) and EPD (eurkaryotic Promoter Database). Several human clones were identified and characterized as alpha, beta and gamma clones as shown in Figure 7. Potential gamma clones were identified on the basis of homology to known rat and mouse sequences. Although

gamma synuclein has been identified in species other than human, this is the first identification of the corresponding gamma synuclein from humans.

Using two primers sets designed from known database sequences (5'ATGTCTTCAAGAAGGGCTTC3'; 5'CCTTGGTCTTCTCAGCTGCT3' and 5'AGCGTGGATGACCTGAAGAG3'; 5'AGCACAGGTGGACAGGCCAAG3'), we have isolated two BAC clones, 139A20 and 174P13, from a Genome System commercial Bacterial Artificial Chromosome library (St. Louis, MO) which contain the human beta and gamma synuclein genes, respectively. The beta gene contained one clone 139A20 has been sequenced as shown in Figure 8 (SEQ ID NO 11), which contains all coding exon sequences and some additional non-coding intronic sequence. The gamma clone 174P13 has been sequenced and is available in GenBank: accession number AF044311. Sequence from the 5' end is given in Figure 9 (SEQ ID NO 12), and sequence from the 3' end is given in Figure 10 (SEQ ID NO 13). The human alpha synuclein gene has also been sequenced as shown in Figure 11, which provides the sequence of each separate exon region with some additional flanking intronic sequence for each exon. (SEQ ID NOs 14-19)

The three human homologues are highly conserved at the protein level. The alpha and beta human homologues have about 60.4% similarity. And the gamma homologue is about 38.3% and 32.8% similar to the alpha and beta homologues, respectively, based on the portion of the coding sequence

that we have obtained thus far. Thus, it is reasonable to presume that mutations in either the beta or gamma synuclein gene may also result in Parkinson's disease.

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44. Gasser et al. (1994) Ann. Neurol. 36: 387.

45. Lynch et al. (1994) Neurology 44: 1878.

46. Jakes et al. (1994) Febs Letts. 345: 27-32.

47. Polymeropoulos et al. (1997) Science 276:2045-2047, which is relied upon and hereby expressly incorporated by reference herein.

48. Lavedan et al. (1998) in press, which is relied upon and hereby expressly incorporated by reference herein.

49. This application is based on provisional application number 60/505,684 filed June 25, 1997 which is relied upon and hereby expressly incorporated by reference herein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Polymeropoulus, Mihael
Lavedan, Christian
Leroy, Elisabeth
Nussbaum, Robert
Johnson, William
Duvoisin, Roger

(ii) TITLE OF INVENTION: Cloning of a gene mutation for Parkinson's disease

(iii) NUMBER OF SEQUENCES: 10

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(B) STREET: 1100 New York Ave. Suite 300 East
(C) CITY: Washington
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(F) ZIP: 20005-3955

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 25-JUN-1998
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Schneller, John W.
(B) REGISTRATION NUMBER: 26,031
(C) REFERENCE/DOCKET NUMBER: NIH 0082A

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (202)414-4000
(B) TELEFAX: (202)414-4040

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS.
(A) LENGTH: 216 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein gene/ exon 4 region

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: 4
(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTAATCAGC AATTTAAGGC TAGCTTGAGA CTTATGTCTT GAATTTGTTT TTGTAGGCTC 60

CAAAACCAAG GAGGGAGTGG TGCATGGTGT GACAACAGGT AAGCTCCATT GTGCTTATAT 120

CAAAGATGAT ATNTAAAGTAT CTAGTGATTA GTGTGGCCCA GTATCAAGAT TCCTATGAA 181

ATTGTAAAACA ATCACTGAGC ATCTAAGAAC ATATC 216

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer #3"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCTAATCAGC AATTTAGGCT AG 22

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer #13"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTATACAAGA ATCTACGAGT C 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(C) INDIVIDUAL ISOLATE: Swiss-Prot P37840

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
35 40 45

Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
50 55 60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
65 70 75 80

Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
85 90 95

Lys Asp Gln Leu Gly Lys Asn Clu Glu Gly Ala Pro Gln Glu Gly Ile
100 105 110

Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
115 120 125

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
130 135 140

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus norvegicus
(C) INDIVIDUAL ISOLATE: Swiss-Prot P37377

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
35 40 45

Val His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
50 55 60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
65 70 75 80

Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Phe Val Lys
85 90 95

Lys Asp Gln Met Gly Lys Gly Glu Glu Gly Tyr Pro Gln Glu Gly Ile
100 105 110

Leu Glu Asp Met Pro Val Asp Pro Ser Ser Glu Ala Tyr Glu Met Pro
115 120 125

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
130 135 140

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 134 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bos taurus
(C) INDIVIDUAL ISOLATE: Swiss-Prot P33567

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val
1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
35 40 45

Val Gln Gly Val Ala Ser Val Ala Glu Lys Thr Lys Glu Gln Ala Ser
50 55 60

His Leu Gly Gly Ala Val Phe Ser Gly Ala Gly Asn Ile Ala Ala Ala
65 70 75 80

Thr Gly Leu Val Lys Lys Glu Glu Phe Pro Thr Asp Leu Lys Pro Glu
85 90 95

Glu Val Ala Gln Glu Ala Ala Glu Glu Pro Leu Ile Glu Pro Leu Met
100 105 110

Glu Pro Glu Gly Glu Ser Tyr Glu Glu Gln Pro Gln Glu Glu Tyr Gln
115 120 125

Glu Tyr Glu Pro Glu Ala
130

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 142 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Serinus canaria
(C) INDIVIDUAL ISOLATE: genbank L33860

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein homologue

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Val Val Ala
1 5 10 15

Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys Thr
20 25 30

Lys Glu Gly Val Leu Tyr Val Gly Ser Arg Thr Lys Glu Gly Val Val
35 40 45

His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Ser Asn
50 55 60

Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys Thr
65 70 75 80

Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Leu Val Lys Lys
85 90 95

Asp Gln Leu Ala Lys Gln Asn Glu Glu Gly Phe Leu Gln Glu Gly Met
100 105 110

Val Asn Asn Thr Gly Ala Ala Val Asp Pro Asp Asn Glu Ala Tyr Glu
115 120 125

Met Pro Pro Glu Glu Glu Tyr Gln Asp Tyr Glu Pro Glu Ala
130 135 140

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Torpedo californica
(C) INDIVIDUAL ISOLATE: Swiss-Prot P37379

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein homologue

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asp Val Leu Lys Lys Gly Phe Ser Phe Ala Lys Glu Gly Val Val
1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Gln Asp Ala Ala Glu Lys
20 25 30

Thr Lys Gln Gly Val Gln Asp Ala Ala Glu Lys Thr Lys Glu Gly Val
35 40 45

Met Tyr Val Gly Thr Lys Thr Lys Glu Gly Val Val Gln Ser Val Asn
50 55 60

Thr Val Thr Glu Lys Thr Lys Glu Gln Ala Asn Val Val Gly Gly Ala
65 70 75 80

Val Val Ala Gly Val Asn Thr Val Ala Ser Lys Thr Val Glu Gly Val
85 90 95

Glu Asn Val Ala Ala Ala Ser Gly Val Val Lys Leu Asp Glu His Gly
100 105 110

Arg Glu Ile Pro Ala Glu Gln Val Ala Glu Gly Lys Gln Thr Thr Gln
115 120 125

Glu Pro Leu Val Glu Ala Thr Glu Ala Thr Glu Glu Thr Gly Lys
130 135 140

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer #1F"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACGACAGTGT GTGTAAAGG 19

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer #13R"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AACATCTGTC AGCAGATCTC 20

(2) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 2809 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 139A20 HUMAN BETA SYNULEIN GENE

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCGCCGCAGC CGCCGCTCCA TCCCCAGCCC CGGCCCCGCA TCCGGTTTGG AAGGGGGCTG
CAAGTTTGCA AGGGGCCCGG GANAAAAANC GAGCAGTGGC CCTTCCCGCG TCCCCAGGGT
TTCAAGGGAC GCTAGGANTN TCCGCGGCCC TGGAGGTTCG CACTGGGGAG TGGGGTGAGA
TGGGGGGAAA GCGGGAGGGG GCTCAGGGTC CAGAAGGGCN CCGCGGTCTC GGGAGTAGGG
GGGCATNTGC GTCCCGCGGG AGGGGCTGGG GTGAGAGTGC GGGGCCAGTG CACCGGTGCC
CGTGTATCGC CCTCCCCAGG CCGCCAGGAT GGACGTGTTC ATGAAGGGCC TGTCCATGGC
CAAGGAGGGC GTTGTGGCAG CCGCGGAGAA AACCAAGCAG GGGGTCACCG AGGCGGCGGA
GAAGACCAAG GAGGGCGTCC TCTACGTCGG TGGGCNGGGG GCNGGGTTTC TGGGGCTGCA
GGGCTGGGGG TCCCCCTACA GTGTGGAGCT GGGGCCGGGT CCCGGGGAGG GGGGTTCTGG
GCAAGATAAT ATNANTCAGC AGATGGGGCN AGGTCANCAN GGGTCATAAG GGACATACCC
ANCCCATAGA ANCCTGGGTC TGTATCCGGA AATGGGGACA CGGGGCGGGC TGATGAGGTG
GGGGGCTCCA NCTGAAAGGC CAGGGACCAN TGCANTNATA AAANCACACA NCCTCCTTTT
TCTTATCTTT TTTACCATTA TTAATAGTTA TCTGGTGTTG AACACTTTCT GTATGCCAAG
TACTGGGTAA AATGTCATAA CATCCATTTC CTCATGTAAT GCTTCCGCCC ATTCTACAGG
TAAGGGAAAC TGGGCTTCCC ATTGGTAGNT AAATTTTAGG TTCAGAAAGG CTTGAATTGA
ATGTCAGTTC AGCCAATTTC TTAGTGGTGG AACCAAACTG AGTTCCATCC GTGAAACGGG
GACAATAACA GCACCCGCTT CCCAGGGCTG GGGAAAAGTG AAGTGCAGCG GGGCAGGCAG
AGGACTTGAC ACAGCACTGG CCCTCAGCCA ACATCCACTA GAGGGGTGGG GTATCGCATC
AGGTGGGAGA GAACTGCAAC CCTTGCAGAC AGAGGTGTGG GGCCCAGTGC AGTGATAAGA
CGGGGGTTAA CATGGGGGTG CAGGTTGTAG GATNTGGGGA CCCAAGGAGG CAGTGACGGG
GCCAGGATGC CCACTCTGTA ATCACCATGC TGTGCTGGAG TTTCTGTTCC CTCAGCGCAG
AGTCCTTAAA TGTGCCGCTT TTTCTNCCCT GCAGGAAGCA AGACCCGAGA AGGTGTGGTA
CAAGGTGTGG CTTCAGGTAC TAGCCCAGCC CTGGCACCAG CCCTTCTCTC AMTTAGGCGG

ATGATCTGGC CGGGAACCAG AGGGCGGGGG CGGGGGAGAC TCCCAAGGCT TCTGCGGGAA

TGCTCCGTGG GGAGGGCAGG CCCTGGGATA CTACAAGGCA GGGCATCGGT GTTTCCCCCT

GGCTCCCAAA CCCCTTCCTC AACCCCCTCC CTGCTCCAGT GGCTGAAAAA ACCAAGGAAC

AGGCCTCACA TCTGGGAGGA GCTGTGTTCT CTGGGGCAGG GAACATCGCA GCAGCCACAG

GACTGGTGAA GAGGGAGGAA TTCCCTACTG ATCTGAAGGT AAGCGATCCT TCTGACCCGC

ACATGCAGGC AAACACACAC ACACACACAC ACACACACCN GGCACACAAA TAAACCTGTC

ACCATCCCCG CCCCCCTAAT CCTGCCACCA GCTTGGAACA CAAGCCACTT TGCCTCCCAT

CCTGCNGGCC CGTGCTAGAC TCAGCTCAGA ATGCATCTGA ATAANGGCGT GCATGGGTGT

GACGCTCCCG GTGATGGGGA CCCAGACCTG GCTGTCTGCG TGTATCCTGC TTGCCAGCGT

GACCCATATG ACTTCTGGCC ACGTCTGCAT GTGTCAATGA TTGTTCATTC ATTTCTTTTC

ATTCAACAAA TATCCATGCC ANANCCAGCC CTGTCCTTGA GCTTCCAGNT CCCTTTCAGC

CNAGGGGAGC NTGAGGGTTA TTTTTGGGGT CCCGATGCCC AGCACAGAGC CTGACACAAA

GGATGAGGCA TAAGCTGGTG ANTGAGTATC CAAATGGTGG AAGTGTGGAG GNTGCCAGGC

ATTGGGGGAG CGGCGTGGAG AGCCAGCTCC CCAATCCATG CTGCCACTTC AACTGTGATT

CGGGGGAATT TCCCCCTTCA CCTCCATCCC ACTTCCAAGG CACTCCAAAT AAATAACTGA

ATTAGAAATT ATCCTTGTTT TGCCAACCCA CCCTAGCCTT CCCCACTCCA ACCCACCCAA

AGCTTACCAC TGTGGGAATT TGGGGGGCAT CCTGGCTGTC CTCACGAGTC CTGACCTTTT

CTGCCCACAG CCAGAGGAAG TGGCCCAGGA AGCTGCTGAA GAACCACTGA TTGAGCCCCT

GATGGAGCCA GAAGGGGAGA GTTATGAGGA CCCACCCCAG GAGGAATATC AGGAGTATGA

GCCAGAGGCG TAGGGGCCCA GGAGAGCCCC CACCAGCAGC ACAATTCTGT CCCTGTCCCT

GCCCCGCCCC CCAGAGCCAG GGCTGTCCTT AGACTCCTTC TCCCCAATCA CGAGATCTTC

CTTCCGCTCT GAGGCAACCC CCTCGGAGCC TGTGTTAGTG TCTGTCCATC TGTCTGTCCT

ACCCGCCCGC GTCCAACCCC GGGGCATGGA CAGGGCCAGG GTTGCGGTCG CGGCTGGGAG

CCTCGCCCCT CCAGTGTTGC CTCCTCCCAT CCAGCGTCTG CGCG

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 223 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 174P13 HUMAN GAMMA SYNULEIN GENE, 5' END

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGGGAGATCC AGCTCCGTCC TGCCTGCAGC AGCACAACCC TGCACACCCA CCATGGATGT

CTTCAAGAAG GGCTTCTCCA TCGCCAAGGA GGGNGTGGTG GGTGCGGTGG AAAAGACCAA

GCAGGGGGTG ACGGAAGCAG CTGAGAAGAC CAAGGAGGGG GTCATGTATG TGGGATTACA

TTTTTTTTTT AAAGAAAGAA TAAATTAATT GTGATTAAAG TTG

(2) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH:677

(B) TYPENUCLEIC ACID

(C) STRANDEDNESS:DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 174P13 HUMAN GAMMA SYNULEIN GENE, 3' END

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTTTTTNAGG GGGGAAAACA GGGAATANAA AAANANGGGG GGGGGTTTTT NNGGGGGGGG

GGGGAAAANG GTTNGGGGGN NAACCNAAAN AAANNCCNAN GGGGGGGGNN ANTNAANTTT

TGGGAACCCA AAGCCCNAGG AGGATTTTTN GTNAANAACG TNACCTCNAG TGGGNCGAGG

AAGACCAAGG AAANGCCCAA CNCGGTTGAN CGAGGCTGTG GTGAACANCG TNCAACNCTG

TGCCCNCCAA NANCGTGGAG GNGGCGGAGA ACATCSCGGT CACCTCCGGG GTGGTGCGCM

AGGAGGACTT GAGGCCATCT KCCCCCCMAC AGGAGGGTGT GGCATCCMAA GARAAAGAGG

AAGTGGCAGA GGAGGCCCAG AGTGGGGGAR ACTAGAGGGC TACAGGCCAG CGTGGATGAC

CTGAAGAGCG CTCCTCTGCC TTGGACACCA TCCCCTCCTA GCACAAGGAG TGCCCGCCTT

GAGTGACATG CGGCTGCCCA CGCTCCTGCC CTCGTCTTCC TGGCCACCCT TGGCCTGTCC

ACCTGTGCTG CTGCACCAAC CTCACTGCCC TCCCTCGGCC CCACCCACCC TCTGGTCCTT

CTGACCCCAC TTATGCTGCT GTGAATTTTT TTTTTAAATG ATTCCAAATA AAACTTGAGC

CCACTCCAAA AAAAAAA

(2) INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 1181 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exons 1 and 2 plus flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AATTTCAGCG ATGCGAGGGC AAAGCGCTCT CGGCGGTGCG GTGTGAGCCA CCTCCCGGCG

CTGCCTGTCT CCTCCAGCAG CTCCCCAAGG GATAGGCTCT GCCCTTGGTG GTCGACCCTC

AGGCCCTCGN TCTCCCAGGN CGACTCTGAC GAGGGGTAGG GGGTGGTCCC CNGGAGGACC

CAGAGGAAAG GCNGGGACAA GAAGGGAGGG GAAGGGGAAA GAGGAAGAGG CATCATCCCT

AGCCCAACCG CTCCCGATCT CCACAAGAGT GCTCGTGACC CTAAACTTAA CGTGAGGCGC

AAAAGCGCCC CAACCTTTTC CCGCCTTGNN CCAGGCAGGC GGCTGGAGTT GATGGCTCAC

CCCGCGCCCC CTGCCCCATC CCCATCCGAG ATAGGGACGA GGAGCACGCT GCAGGGAAAG

CAGCGAGCGC CGGGAGAGGG GCGGGCAGAA GCGCTGACAA ATCAGCGGTG GGGGCGGAGA

GCCGAGGAGA AGGAGAAGGA GGAGGACTAG GAGGAGGAGG ACGGCGACGA CCAGAAGGGG

CCCAAGAGAG GGGGCGAGCG ACCGAGCGCC GCGACGCGAA GTGAGGTGCG TGCGGGCTCA

GCGCAGACCC CGGCCCGGCC CCTCCTGAGA GCGTCCTGGG CGCTCCCTCA CGCCTTGCCT

TCAAGCCTTC TGCCTTTCCA CCCTCGTGAG CGGAGAACTG GGAGTGGCCA TTCGACGACA

GGTTAGCGGG TTTGCCTCCC ACTCCCCCAG CCTCGCGTCG CCGGCTCACA GCGGCCTCCT

CTGGGGACAG TCCCCCCCGG GTGCCCCTCC GCCCTTCCTG TGCGCTCCTT TTCCTTCTTC

TTTCCTATTA AATATTATTT GGGAATTGTT TAAATTTTTT TTTTAAAAAA AGAGAGAGGC

GNGGAGGAGT CGGAGTTGTG GAGAAGCAGA GGGACTCAGG TAAGTACCTG TGGATCTAAA

CGGGNGTCTT TTGGAAATCC TGGAGAACGC CGGATGGAGA CGAATGGTCG TGGGNACCGG

GAGGGGGTGG TGCTGCCATG AGGACCGCTG GGCCAGGTCT CTGGGAGGTG AGTACTTGTC

CTTTGGGGAG CTAAGGAAAG AGACTTGACC TGGCTTTCGT CCTGCTTCTG ATATTCCCTT

CTCCACAAGG GCTGAGAGNT TAGGCTGCTT CTCCGGGATC C

(2) INFORMATION FOR SEQ ID NO:15

(i)SEQUENCE CHARACTERISITCS

(A)LENGTH: 536 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exon 3 plus flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTTAAAAGAG TCTCACACTT TGGAGGGTTT CTCATGATTT TTCAGTGTTT TTTGTTTATT

TTTCCCCGAA AGTTCTCATT CAAAGTGTAT TTTATGTTTT CCAGTGTGGT GTAAAGAAAT

TCATTAGCCA TGGATGTATT CATGAAAGGA CTTTCAAAGG CCAAGGAGGG AGTTGTGGCT

GCTGCTGAGA AAACCAAACA GGGTGTGGCA CAAGCAGCAG GAAAGACAAA AGAGGGTGNT

CTCTATGTAG GTAGGTAAAC CCCAAATGTC AGTTTGGTGC TTGTTCATGA GTGATGGGTT

AGGATAACAA TACTCTAAAT GCTGGTAGTT CTCTCTCTTG ATTCATTTTT GCATCATTGC

TTGTCAAAAA GGTGGACTGA GTCAGAGGTA TGTGTAGGTA GGTGAATGTG AACGTGTGTA

TNTGAGCTAA TAGTAAAAAT GCGACTGTTT GCTTTTCAGA TTTTTAATTT TGCCTAATAT

NTATGACTTN TTAAAATGAA TGTTTCTGTA CTACATAATT CTATNTCAGA GACAGT

(2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 650 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exon 4 plus flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGCAGGTCA ACGGATCTGT CTCTAGTGCT GTACTTTTAA AGCTTCTACA GTTCTGAATT

CAAAATTATC TTCTCACTGG GCCCCGGTGT TATCTCATTC TTTTTTCTCC TCTGTAAGTT

GACATGTGAT GTGGGAACAA AGGGGATAAA GTCATTATTT TGTGCTAAAA TCGTAATTGG

AGAGGACCTC CTGTTAGCTG GGCTTTCTTC TATNTATTGT GGTGGTTAGG AGTTCCTTCT

TCTAGTTTTA GGATATATAT ATATATTTTT TCTTTCCCTG AAGATATAAT AATATATATA

CTTCTGAAGA TTGAGATTTT TAAATTAGTT GTATTGAAAA CTAGCTAATC AGCAATTTAA

GGCTAGCTTG AGACTTATGT CTTGAATTTG TTTTTGTAGG CTCCAAAACC AAGGAGGGAG

TGGTGCATGG TGTGGCAACA GGTAAGCTCC ATTGTGCTTA TATCAAAGAT GATATNTAAA

GTATCTAGTG ATTAGTGTGG CCCAGTATCA AGATTCCTAT TGAAATTGTA AAACAATCAC

TGAGCATCTA AGAACATATC AGTCTTATTG AAACTGAATT CTTTATAAAG TATTTTTAAA

TAGGTAAATA TTGATTATAA ATAAAAAATA TACTTGCCAA GAATAATGAG

(2) INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 504 base pairs

(B)TYPE: NUCLEIC ACID

(C)STRANDEDNESS:DOUBLE

(D)TOPOLOGY: LINEAR

(ii)MOLECULAR TYPE: DNA (genomic)

(iii)HYPOTHETICAL: NO

(iv)ANTI-SENSE: NO

(vii)IMMEDIATE SOURCE:

(A)CLONE: human alpha synuclein gene/ exon 5 plus flanking intron sequences

(viii)POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATATCTTAGC CAAGATTCAA TGTTTGGTTG AACCACACTC ACTTGACATC TTGGTGGCTT

TTGTTTCTTC TGACCACTCA GTTATCTATG GCATGTGTAG ATACAGGTGT ATGGAANCGA

TGGCTAGTGG AAGTGGAATG ATTTTAAGTC ACTGTTATTC TACCACCCTT TAATCTGTTG

TTGCTCTTTA TTTGTACCAG TGGCTGAGAA GACCAAAGAG CAAGTGACAA ATGTTGGAGG

AGCAGTGGTG ACGGGTGTGA CAGCAGTAGC CCAGAAGACA GTGGAGGGAG CAGGGAGCAT

TGCAGCAGCC ACTGGCTTTG TCAAAAAGGA CCAGTTGGGC AAGGTATGGC TGTGTACGTT

TTGTGTTACA TTTATAAGCT GGTGAGATTA CGGTTCATTT TCATGTGAAG CCTGGAGGCA

GGAGCAAGAT ACTTACTGTG GGGAACGGCT ACCTGACCCT CCCCTTGTGA AAAAGTGCTA

CCTTTATATT GGTCTTGCTT GTTT

(2) INFORMATION FOR SEQ ID NO:18

(i)SEQUENCE CHARACTERISITCS

(A)LENGTH: 727 base pairs

(B)TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exons 1 and 2 plus flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AAAAGTTTAC ATACTTTGAG GTTGATAACC CATGTTGCCG CAATGTTTCC CCGGAGGCAT
TGTGGAGTTT AGAATGCCAG TAGTAATATT AAGGTGTGCC ATTTTCAAGA TCCGTGGCCA
ACATCCCTAT ATGTAAGATT TTTCCAAAAC ATGGTTCTGA TTTTTAAAAG TGAAAAATGC
TACTTCATCA TGTTCTTTTT GTGCTTCTTA CTTTAAATAT TAGAATGAAG AAGGAGCCCC
ACAGGAAGGA ATTCTGGAAG ATATGCCTGT GGATCCTGAC AATGAGGCTT ATGAAATGCC
TTCTGAGGTA GGAGTCCAAG CTGAATCTTT CTAACAAGAC AGTACCAAAA ACCTGTCATT
GTCACATTTC TCTTTCATTA GTGCTTAGTG AGAATCATTT GCTCTCTACA TGCTCATTA
GTGGACAACT TGCAAGTTAA GAATAGTTTT TACATTTTTA AAGGGTCCTT AAAAAAAAAG
AGGAGGAGGA AGATGAAGAA GAGGAAGAAA GGATGTAAAA GAAATCATAT GTAGTCCACA
TAGCTTAATA TACNTACTAC TTGACCCTTT ACAGGAAAAG CTTTACTAAC CCCTGCATTA
GAGAATATAT TTTTTTGCAA AAACATTGAT TGTAAATTTT AGTGTAAAGT GGGGAGCCAT
TTCCTATCTC ATTGGCTGTC CAGTGCTGAT GCGTAATTGA AACTTATACT AACAGTGTGT
GCTGTCT

(2) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 1596 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exon 7 plus flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTTTGATTTT TCTAATATTA GGAAGGGTAT CAAGACTACG AACCTGAAGC CTAAGAAATA

TCTTTGCTCC CAGTTTCTTG AGATCTGCTG ACAGATGTTC CATCCTGTAC AAGTGCTCAG

TTCCAATGTG CCCAGTCATG ACATTTCTCA AAGTTTTTAC AGTGTATCTC GAAGTCTTCC

ATCAGCAGTG ATTGAAGCAT CTGTACCTGC CCCCACTCAG CATTTCGGTG CTTCCCTTTC

ACTGAAGTGA ATACATGGTA GCAGGGTCTT TGTGTGCTGT GGATTTTGTG GCTTCAATCT

ACGATGTTAA AACAAATTAA AAACACCTAA GTGACTACCA CTTATTTCTA AATCCTCACT

ATTTTTTTGT TGCTGTTGTT CAGAAGTTGT TAGTGATTTG CTATCATATA TTATNAGATT

TTTAGGTGTC TTTTAATGAT ACTGTCTAAG AATAATGACG TATTGTGAAA TTTGTTAATA

TATATNATAC TTAAAAATAT GTGAGCATGA AACTATGCAC CTATAATACT AAATATGAAA

TTTTACCATT TTGCGATGTG TTTTATTCAC TTGTGTTTGT ATATNAATGG TGAGAATTAA

AATAAAACGT TATCTCATTG CAAAAATATT TTATTTTTAT CCCATCTCAC TTTAATAATA

AAAATCATGC TTATAAGCAA CATGAATTAA GAACTGACAC AAAGGACAAA AATATAAAGT

TATTAATAGC CATTTGAAGA AGGAGGAATT TTAGAAGAGG TAGAGAAAAT GGAACATTAA

CCCTACACTC GGAATTCCCT GAAGCAACAC TGCCAGAAGT GTGTTTTGGT ATGCACTGGT

TCCTTAAGTG GCTGTGATTA ATTATTGAAA GTGGGGTGTT GAAGACCCCA ACTACTATTG

TAGAGTGGTC TATTTCTCCC TTCAATCCTG TCAATGTTTG CTTTACGTAT TTTGGGGAAC

TGTTGTTTGA TGTGTATGTG TTTATAATTG TTATACATTT TTAATTGAGC CTTTTATTAA

CATATATTGT TATTTTTGTC TCGAAATAAT TTTTTAGTTA AAATCTATTT TGTCTGATAT

TGGTGTGAAT GCTGTACCTT TCTGACAATA AATAATATNC GACCATGAAT AAAAAAAAAA

AAAAAGTGGG TTCCCGGGAA CTAAGCAGTG TAGAAGATGA TTTTGACTAC ACCCTCCTTA

GAGAGCCATA AGACACATTA GCACATATTA GCACATTCAA GGCTCTGAGA GAATGTGGTT

AACTTTGTTT AACTCAGCAT TCCTCACTTT TTTTTTTTAA TCATCAGAAA TTCTCTCTCT

CTCTCTCTTT TTCTCTCGCT CTCTTTTTTT TTTTTTTTTT TTTTACAGGA AATGCCTTTA

AACATCGTTG GGAACTACCA GAGTCACCTT AAAGGGAGNA TCAATTCTCT AGGACTGGAT

AAAAATTTCA TGGGCCTCCT TTAAAATGTT GCCCAAATAT ATGGAATTCT AGGGGTTTTT

CCNTAGGGGG AAGGGTTTTT TCTCTTTTCN GGGGAGGATC CTTTTAACNC CCCNGGGGGG

NGCCCGGAAA ATAAACTTGG NGGGGGGGNA AAACTT

WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising a nucleotide sequence encoding a mutated human synuclein protein or homologue thereof.

2. The isolated nucleic acid of claim 1 wherein said mutated synuclein protein is selected from the group consisting of alpha, beta and gamma synuclein proteins.

3. The isolated nucleic acid of claim 2 wherein said mutated synuclein protein is the alpha synuclein protein.

4. The isolated nucleic acid of claim 3 wherein said nucleotide sequence contains at least one mutation at base pair position 209.

5. The isolated nucleic acid of claim 4 wherein said mutation at position 209 is a change from guanine to adenine.

6. The isolated nucleic acid of claim 5 having the sequence given in SEQ ID NO. 1.

7. An oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.

8. The oligonucleotide of claim 7 wherein said mutation is at base pair position 209 in the synnuclein gene.

9. The oligonucleotide of claim 8 wherein said mutation is a change from guanine to adenine.

10. A vector comprising the isolated nucleic acid of claim 1.

11. A host cell comprising the vector of claim 10.

12. A method of affecting characteristics of Parkinson's Disease, comprising expressing nucleic acids which are implicated in disease development in cultured cells through the use of expression vectors.

13. The method of claim 12 wherein the said nucleic acid is selected from the group consisting of alpha, beta, and gamma synuclein genes.

14. The method of claim 13 wherein the said nucleic acid encodes the mutated alpha synuclein protein.

15. The method in claim 14 wherein the said mutated alpha synuclein protein contains at least one mutation at base pair 209.

16. The method of claim 15 wherein said mutation at position 209 is a change from guanine to adenine.

17. An isolated human synuclein protein or peptide containing at least one mutation.

18. The isolated human synuclein protein or peptide of claim 17 wherein said protein or peptide is selected from the group consisting of

the human alpha, beta and gamma synuclein proteins or fragments thereof.

19. The isolated human synuclein protein or peptide of claim 18 having the sequence given in SEQ ID NO 5.

20. The isolated human synuclein protein or peptide of claim 19 wherein said protein or peptide is the alpha synuclein gene or a fragment thereof.

21. The isolated protein or peptide of claim 20, wherein said mutation is at amino acid position 53.

22. The isolated protein or peptide of claim 21, wherein said mutation is an alanine to threonine substitution.

23. An antibody specific for the protein or peptide of claim 17.

24. A method of detecting subjects at increased risk for Parkinson's Disease, comprising:

obtaining a sample comprising nucleic acids, proteins or tissues from the subjects, and

detecting in the nucleic acids, proteins or tissues the presence of a mutation which is associated with Parkinson's disease,

thereby identifying subjects at increased risk for the disease.

25. The method of claim 24 wherein said mutation is located on human chromosome four.

26. The method of claim 25 wherein said mutation is located in the alpha synuclein gene.

27. The method of claim 26 wherein said mutation causes an amino acid substitution at position 53.

28. The method of claim 27 wherein said mutation causes an alanine to threonine substitution at position 53.

29. The method of claim 24 wherein said detecting step comprises combining a nucleotide probe which selectively hybridizes to a nucleic acid containing said mutation, and detecting the presence of hybridization.

30. The method of claim 29 wherein said nucleotide probe is an oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.

31. The method of claim 30 wherin the mutation of said oligonucleotide is at base pair position 209 in the alpha synuclein gene.

32. The method of claim 31 wherein the mutation of said oligonucleotide is a change from guanine to adenine.

33. The method of claim 24 wherein said detecting step comprises amplifying a nucleic acid product comprising said mutation, and detecting

the presence of said mutation in the amplified product.

34. The method of claim 33 wherein said detecting step comprises selectively amplifying a nucleic acid product comprising said mutation, and detecting the presence of amplification.

35. The method of claim 34 wherein said amplifying step comprises at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids.

36. The method of claim 35 wherein said amplifying step uses two oligonucleotides.

37. The method of claim 36 wherein said two oligonucleotides have the sequences of SEQ ID NOs 2 and 3.

38. The method of claim 24 wherein said detecting step comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of said sample of nucleic acids.

39. The method of claim 38 wherein said restriction endonuclease site is recognized by *Tsp*451.

40. The method of claim 24 wherein said detecting step comprises chain termination with a labeled dideoxynucleotide.

41. An oligonucleotide complementary to a nucleic acid sequence

which flanks a mutation in the synuclein gene that is asscciated with predisposition to Parkinson's disease, wherein said oligonucleotide may be used in diagnostic screens in the amplification of a nucleic acid sequence comprising said mutation.

42. The oligonucleotide of claim 41 having the sequence of SEQ ID NO 2.

43. The oligonucleotide of claim 41 having the sequence of SEQ ID NO 3.

44. The method of claim 24 wherein said detection step comprises identification of said mutations with an antibody.

45. The method of claim 44 wherein said antibody is directed against an isolated human synuclein protein or peptide containing at least one mutation.

46. The method of claim 45 wherein said isolated human synuclein protein or peptide is selected from a group consisting of the human alpha, beta, and gamma synuclein proteins or fragments thereof.

47. The method of claim 46 wherein said isolated human synuclein protein or peptide has the mutated sequence given in SEQ ID NO 5.

48. The method of claim 47 wherein said mutation is at amino acid position 53.

49. The method of claim 48 wherein said mutation is an alanine to threonine substition

50. A diagnostic kit comprising the oligonucleotide of claim 41.

51. A diagnostic kit comprising the oligonucleotide of claim 42.

52. A diagnostic kit comprising the oligonucleotide of claim 43.

53. A diagnostic kit comprising the oligonucleotide of claim 7.

54. A diagnostic kit comprising the oligonucleotide of claim 8.

55. A diagnostic kit comprising the oligonucleotide of claim 9.

56. A diagnostic kit comprising the antibody of claim 23.

57. An isolated nucleic acid comprising a mutation in a human synuclein gene or homologue thereof.

58. The isolated nucleic acid of claim 57 wherein said synuclein gene is the alpha synuclein gene.

59. The isolated nucleic acid of claim 58 wherein said mutation occurs at base pair position 209.

60. The isolated nucleic acid of claim 59 wherein said mutation is

a change from guanine to adenine.

61. The isolated nucleic acid of claim 60 having the sequence given in SEQ ID NO 1.

62. A transgenic animal which expresses a mutated synuclein protein, wherein said animal may be used as an animal model for Parkinson's disease.

63. The transgenic animal of claim 62, wherein said mutated synuclein protein is an alpha synuclein protein.

64. A method of screening a compound for the ability to reverse the self-aggregation of synuclein proteins, comprising exposing an aggregate of synuclein proteins to a test compound and observing whether or not the aggregate is dissolved.

65. The method of claim 64 wherein said test compound is a synuclein peptide.

66. The method of claim 65 wherein said peptide comprises a mutation.

67. The method of claim 64 wherein said test compound is an antibody.

68. The method of claim 64, wherein said observing step comprises

Congo red staining, electron microscopy or CD spectrometry.

69. The method of claim 64 wherein said protein aggregate is located within an animal.

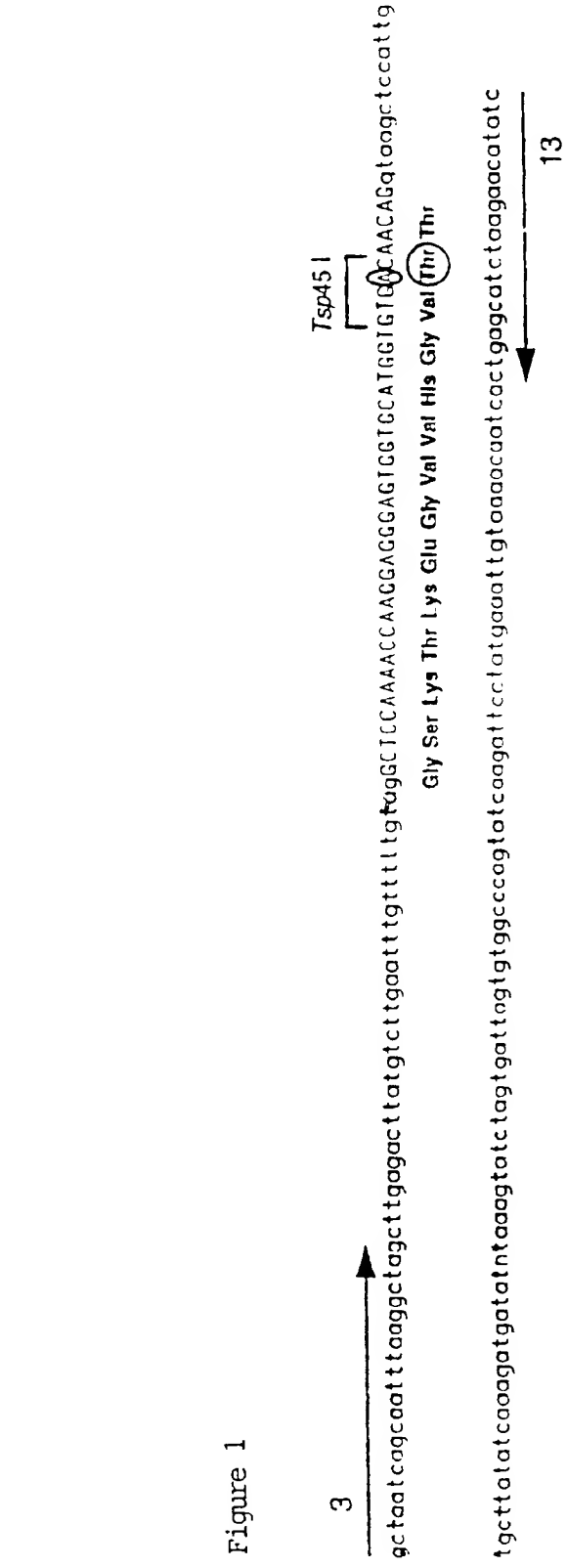
70. A method of screening a compound for the ability to inhibit the self-aggregation of synuclein proteins, comprising exposing a population of synuclein proteins to a test compound under conditions which promote self-aggregation in the absence of said compound and observing whether or not self-aggregation of said proteins is inhibited.

71. The method of claim 70 wherein said test compound is a synuclein peptide.

72. The method of claim 71 wherein said peptide comprises a mutation.

73. The method of claim 70 wherein said test compound is an antibody.

74. The invention substantially as disclosed and described.


Tsp451
3
13
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Gly Ser Lys Thr Lys Glu Gly Val Val His Gly Val Thr Thr
tgcttatatcaaagatgatatntaaagtatctagtgattagtgtggcccagtatcaagattcctatgaaattgtaaaacaatcactgagcatctaaggaacatatc

Figure 1

Figure 2

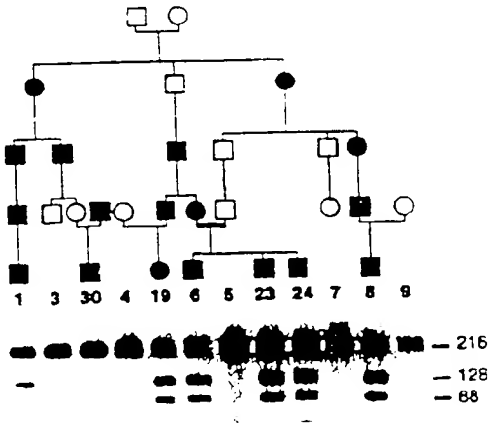


Figure 3

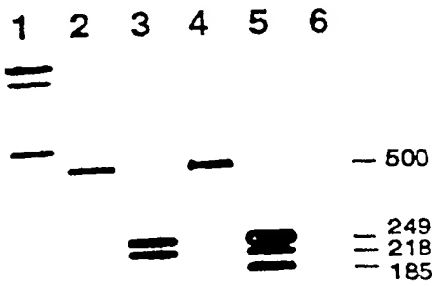
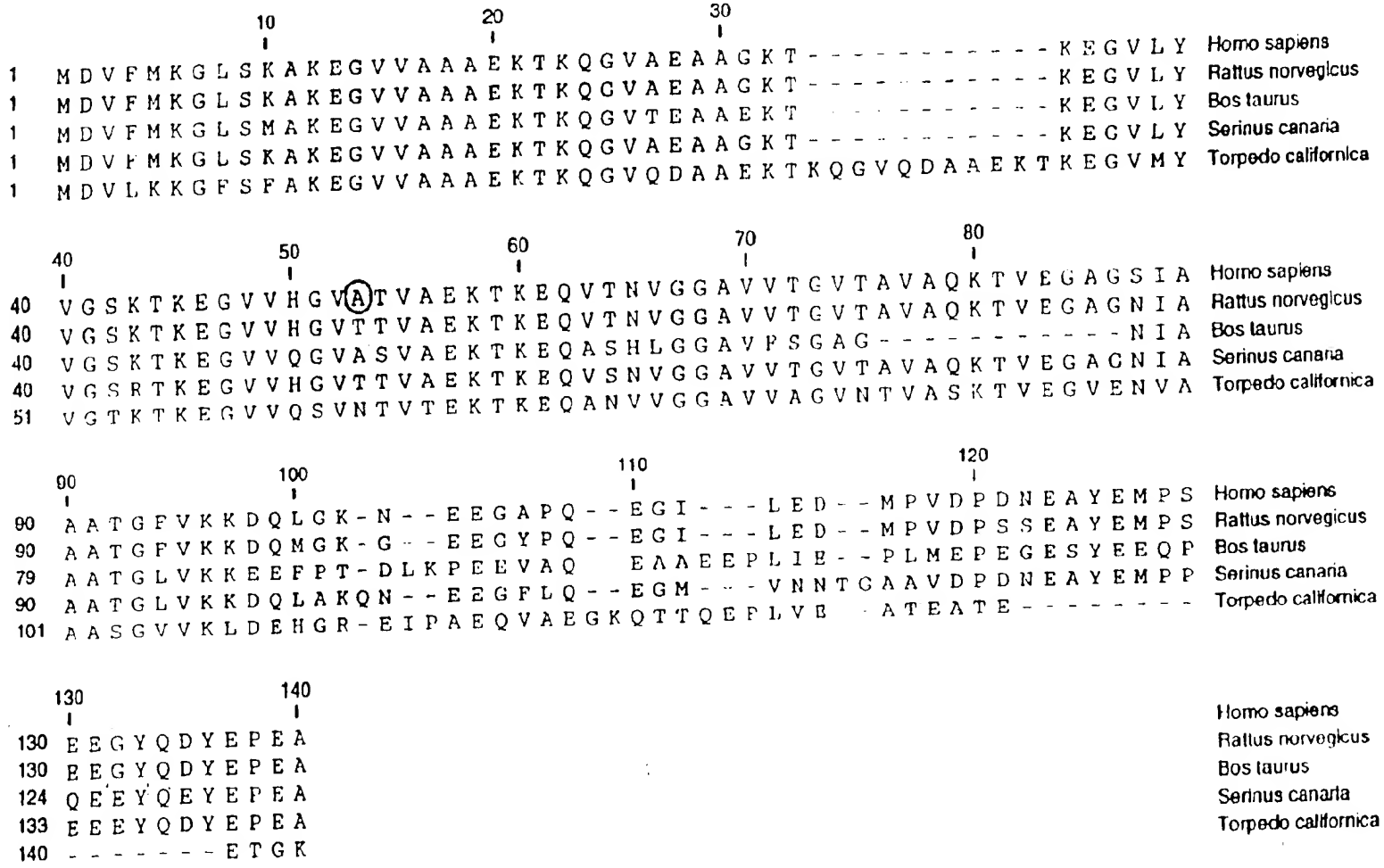


Figure 4


10 20 30
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1 MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKT----------KEGVLY Rattus norvegicus
1 MDVFMKGLSKAKEGVVAAAEKTKQGVTEAAEKT----------KEGVLY Bos taurus
1 MDVFMKGLSMAKEGVVAAAEKTKQGVAEAAGKT----------KEGVLY Serinus canaria
1 MDVFMKGLSKAKEGVVAAAEKTKQGVQDAAEKTKQGVQDAAEKTKEGVMY Torpedo californica
40 50 60 70 80
40 VGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIA Homo sapiens
40 VGSKTKEGVVHGVTTVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGNIA Rattus norvegicus
40 VGSKTKEGVVQGVASVAEKTKEQASHLGGAVFSGAG----------NIA Bos taurus
40 VGSRTKEGVVHGVTTVAEKTKEQVSNVGGAVVTGVTAVAQKTVEGAGNIA Serinus canaria
51 VGTKTKEGVVQSVNTVTEKTKEQANVVGGAVVAGVNTVASKTVEGVENVA Torpedo californica
90 100 110 120
90 AATGFVKKDQLGK-N--EEGAPQ--EGI---LED--MPVDPDNEAYEMPS Homo sapiens
90 AATGFVKKDQMGK-G--EEGYPQ--EGI---LED--MPVDPSSEAYEMPS Rattus norvegicus
90 AATGFVKKDQMGK-G--EEGYPQ--EGI---LED--PLMEPEGESYEEQP Bos taurus
79 AATGLVKKEEFPT-DLKPEEVAQ EAAEEPLIE--PLMEPEGESYEEQP Serinus canaria
90 AATGLVKKDQLAKQN--EEGFLQ--EGM---VNNTGAAVDPDNEAYEMPP Torpedo californica
101 AASGVVKLDEHGR-EIPAEQVAEGKQTTQEPLVE ATEATE--------- Torpedo californica
130 140
130 EEGYQDYEPEA Homo sapiens
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140 -------ETGK Torpedo californica

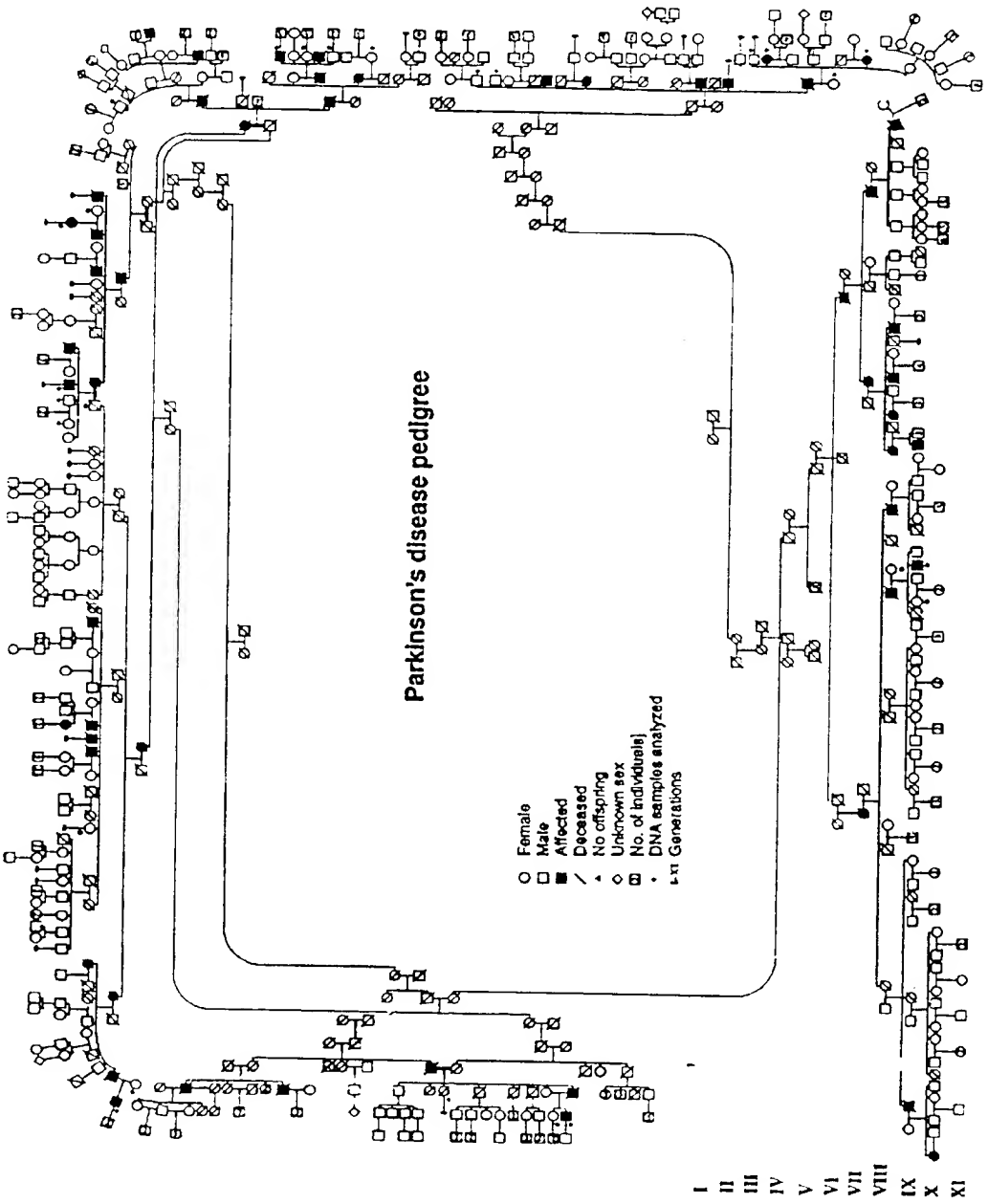
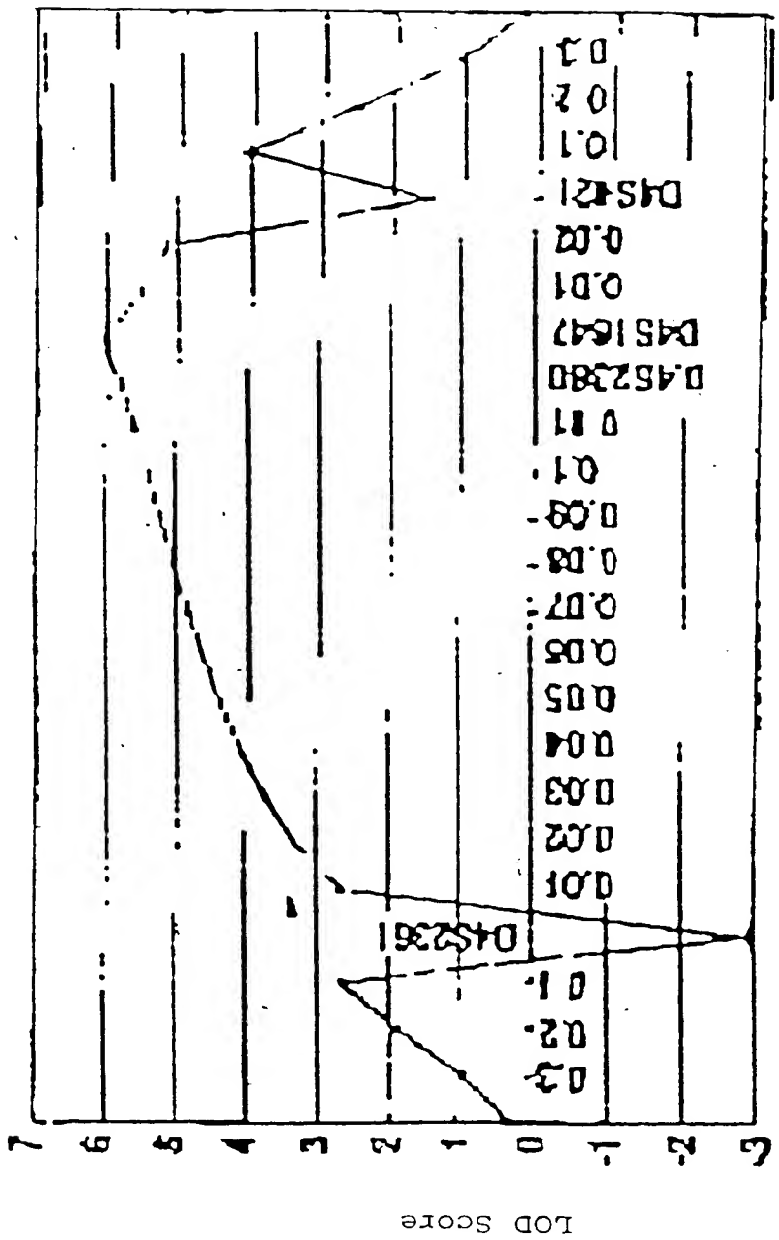

Parkinson's disease pedigree
Female
Male
Affected
Deceased
No offspring
Unknown sex
No. of individuals
DNA samples analyzed
Generations
I
II
III
IV
V
VI
VII
VIII
IX
X
XI

Figure 5


LOD Score
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6
5
4
3
2
1
0
1
2
3
0.3
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0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
D4S2361
0.1
0.2
0.3

Figure 6

Figure 7

clone	5'	3'	gene
109979	T84229	T88834	alpha
111088	T83410		alpha
111090	T83411	T81593	alpha
130048	R11619	(R19409)	alpha
135534	R31354	R32856	alpha
141246	R66863	R67383	alpha
145594	R78091	R77746	alpha
171906	H19290	H19291	beta
172284	H19556	H19474	beta
172749		H19685	beta
175546		H41126	beta
193174	H47503	H47504	alpha
210768	H66914	H66869	alpha
213616	H70324	H70325	alpha
236027	H62070		alpha
248153	N53829	N73325	alpha
24991	(T80528)	R39000	alpha
26298	R13508	(R20629)	alpha
265817	N28661	N21457	alpha
266628		N22757	alpha
27342		R37173	alpha
280344	(N50305)	N47094	alpha
290894		N72005	alpha
294142		N68597	alpha
307787	W21278		alpha
340635	W56712	W56757	alpha
340683	W55986	W56276	alpha
346647	W94390	W74638	alpha
346796	W79585	W79784	alpha
359349	AA010546	AA010547	alpha
364632	AA022809	AA022690	alpha
39915		R50455	beta
40764	R56327	R56245	alpha
45086	H08908	H08824	alpha
48607	H10267	H10213	alpha
49811	H29080	H28976	alpha
50202		H17962	beta
50470		H16811	beta
66473	R16018	R16119	alpha
667794	AA258686	AA258608	alpha
69907	T48654	T48655	alpha
72391	AA394097	AA293803	gamma
739009	AA421586		beta
739014	(AA42185)	AA421567	beta
771303		AA443638	gamma
2-4		L36675	alpha
2-5		L36674	alpha
c-01f06		F01363	alpha
c-1rb08	F03254	F06981	alpha
c-2td12	F08836	F11169	alpha
c-28f08	F03751	F07521	alpha
cDNA	S69965		beta
EST01420 (HRBAA27)	M78265		gamma
EST19193	AA317129		beta
EST22040	AA319774		alpha

Figure 7 cont.

EST26845	T28079		beta
EST31489	AA328063		alpha
EST68G11	W22518		gamma
F1-625D	R29481		alpha
GEN-129D09	D61090		beta
hbc590	T11070		alpha
HIBBA65	T08213	T08212	alpha
	HR70E3R	HR70E3F	alpha
HSNACP0		U46896-46901	alpha
KK1311	N83633		alpha
		D318839	alpha
		L08850	alpha
	T28735		alpha
	Z20502		alpha

Figure 8


10 20 30 40 50 60 70
CCGCCGCAGCCGCCGCTCCATCCCCAGCCCCGGCCCCGCATCCGGTTTGGAAGGGGGCTGCAAGTTTGCA 70
AGGGGCCCGGGAXAAAAAXCGAGCAGTGGCCCTTCCCGCGTCCCCAGGGTTTCAAGGGACGCTAGGAXTX 140
TCCGCGGCCCTGGAGGTTCGCACTGGGGAGTGGGGTGAGATGGGGGGAAAGCGGGAGGGGGCTCAGGGTC 210
CAGAAGGGCXCCGCGGTCTCGGGAGTAGGGGGGCATXTGCGTCCCGCGGGAGGGGCTGGGGTGAGAGTGC 280
GGGGCCAGTGCACCGGTGCCCGTGTATCGCCCTCCCCAGGCCGCCAGGATGGACGTGTTCATGAAGGGCC 350
360 370 380 390 400 410 420
TGTCCATGGCCAAGGAGGGCGTTGTGGCAGCCGCGGAGAAAACCAAGCAGGGGGTCACCGAGGCGGCGGA 420
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710 720 730 740 750 760 770
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CAGTGACGGGGCCAGGATGCCCACTCTGTAATCACCATGCTGTGCTGGAGTTTCTGTTCCCTCAGCGCAG 1260
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CITCAGGTACTAGCCCAGCCCTGGCACCAGCCCTTCTCTCAMTTAGGCGGATGATCTGGCCGGGAACCAG 1400
1410 1420 1430 1440 1450 1460 1470
AGGGCGGGGGCGGGGGAGACTCCCAAGGCTTCTGCGGGAATGCTCCGTGGGGAGGGCAGGCCCTGGGATA 1470
CTACAAGGCAGGGCATCGGTGTTTCCCCCTGGCTCCCAAACCCCTTCCTCAACCCCCTCCCTGCTCCAGT 1540
GGCTGAAAAAACCAAGGAACAGGCCTCACATCTGGGAGGAGCTGTGTTCTCTGGGGCAGGGAACATCGCA 1610
GCAGCCACAGGACTGGTGAAGAGGGAGGAATTCCCTACTGATCTGAAGGTAAGCGATCCTTCTGACCCGC 1680
ACATGCAGGCAAACACACACACACACACACACACACACACCXGGCACACAAATAAACCTGTCACCATCCCCG 1750
1760 1770 1780 1790 1800 1810 1820
CCCCCCTAATCCTGCCACCAGCTTGGAACACAAGCCACTTTGCCTCCCATCCTGCXGGCCCGTGCTAGAC 1820
TCAGCTCAGAATGCATCTGAATAAXGGCGTGCATGGGTGTGACGCTCCCGGTGATGGGGACCCAGACCTG 1890
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2460 2470 2480 2490 2500 2510 2520
TTGAGCCCCTGATGGAGCCAGAAGGGGAGAGTTATGAGGACCCACCCCAGGAGGAATATCAGGAGTATGA 2520
GCCAGAGGCGTAGGGGCCCAGGAGAGCCCCCACCAGCAGCACAATTCTGTCCCTGTCCCTGCCCCGCCCC 2590
CCAGAGCCAGGGCTGTCCTTAGACTCCTTCTCCCCAATCACGAGATCTTCCTTCCGCTCTGAGGCAACCC 2660
CCTCGGAGCCTGTGTTAGTGTCTGTCCATCTGTCTGTCCTACCCGCCCGCGTCCAACCCCGGGGCATGGA 2730
CAGGGCCAGGGTTGCGGTCGCGGCTGGGAGCCTCGCCCCTCCAGTGTTGCCTCCTCCCATCCAGCGTCTG 2800
2810 2820 2830 2840 2850 2860 2870
CGCG 2804

Figure 9

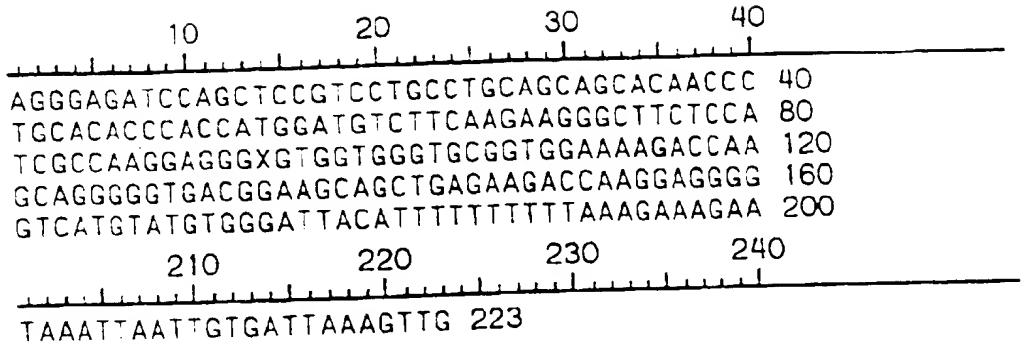

Figure 10

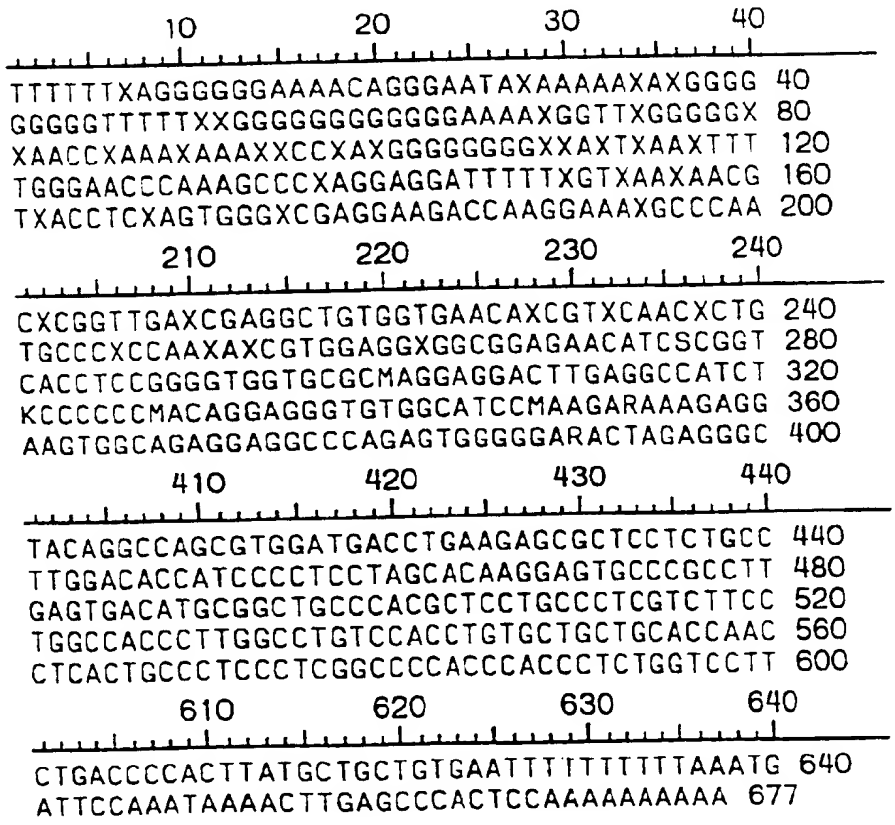

Figure 11

alpha-SYN exons 1-2

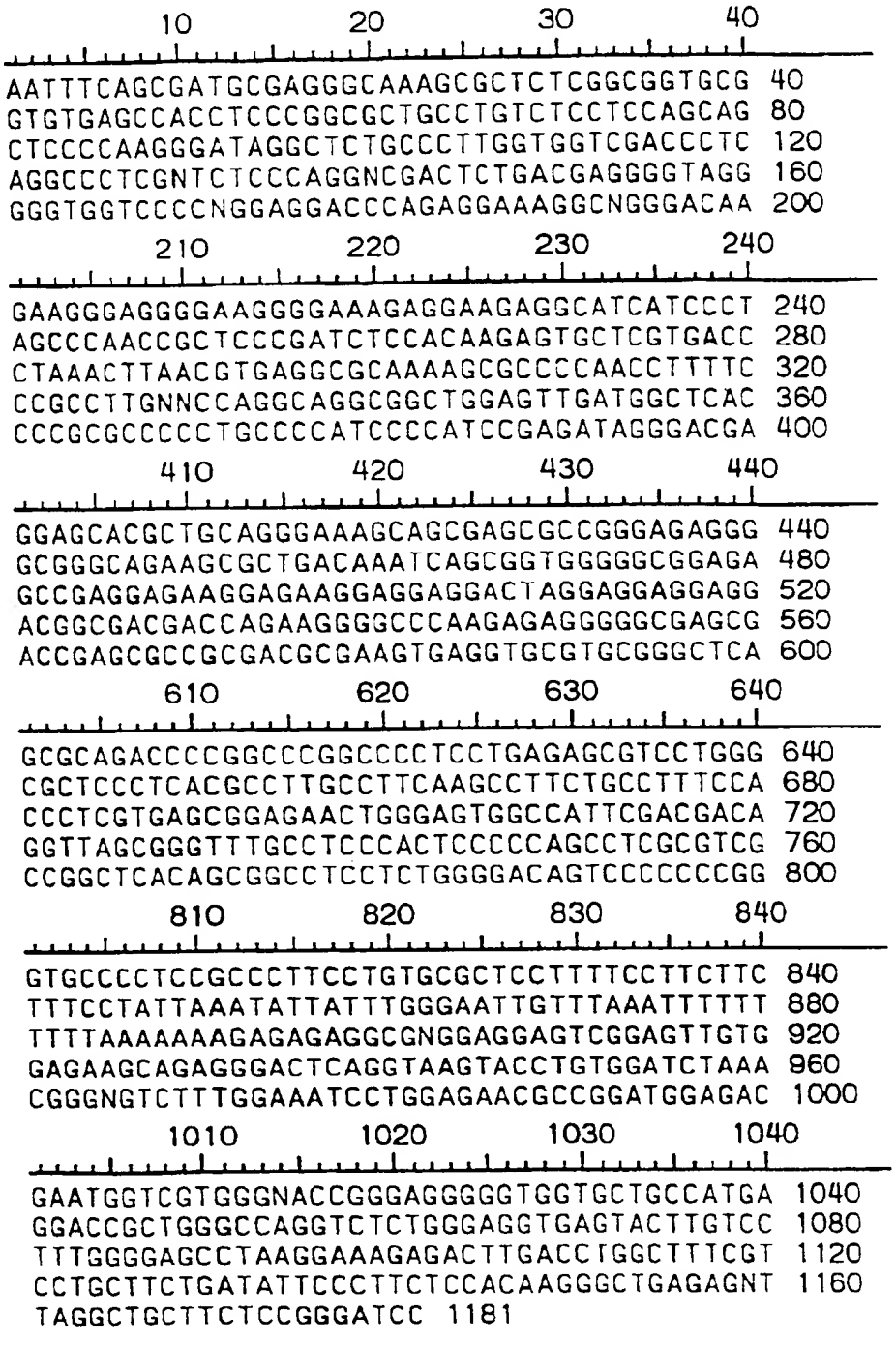

Figure 11 cont.

alpha-SYN exon 3

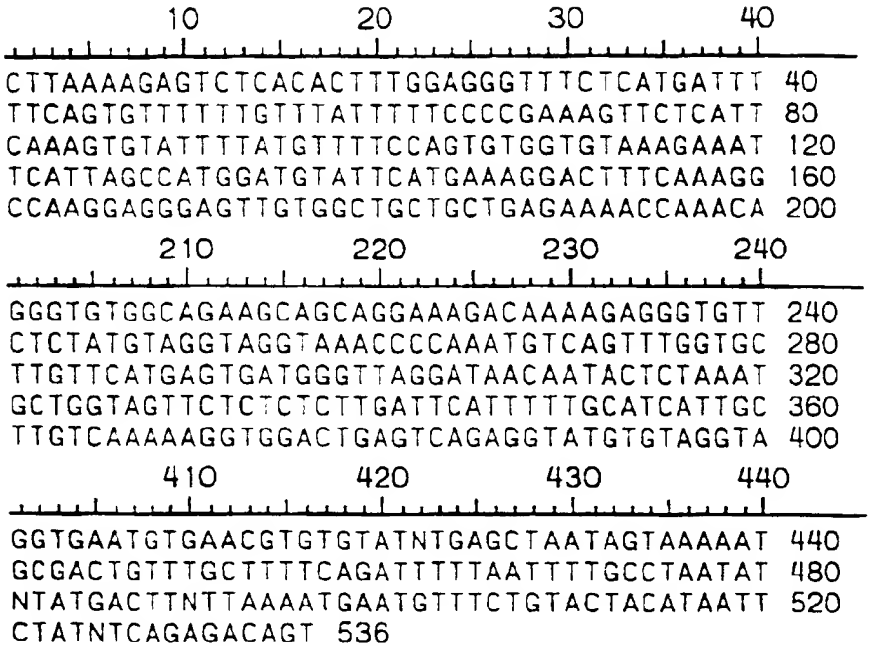

Figure 11 cont.

alpha-SYN exon 4

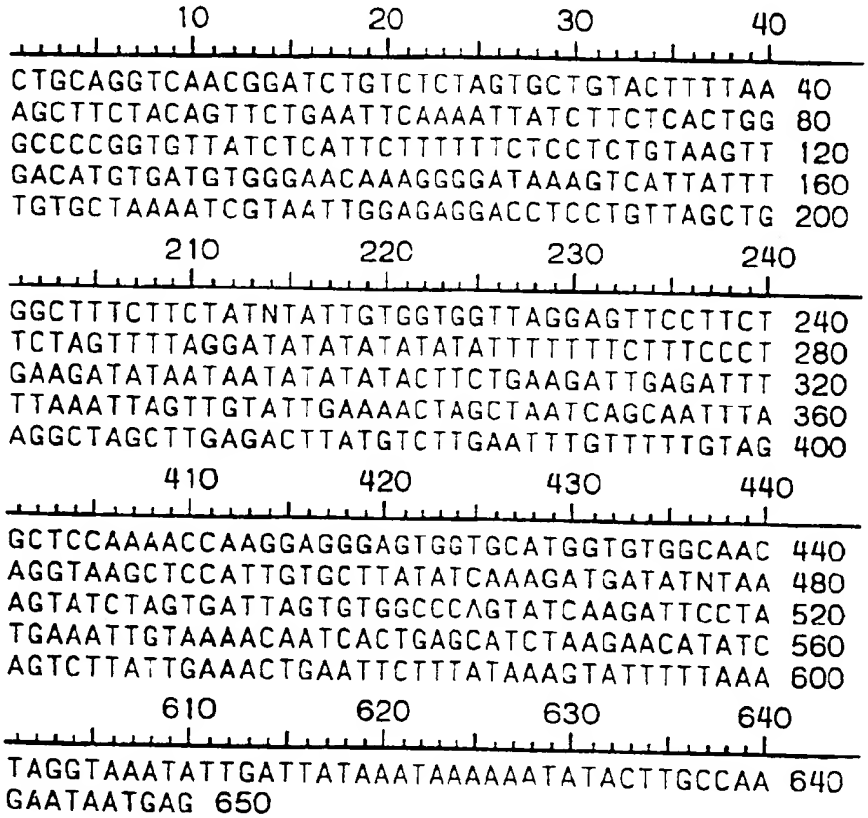

Figure 11 cont.

alpha-SYN exon 5

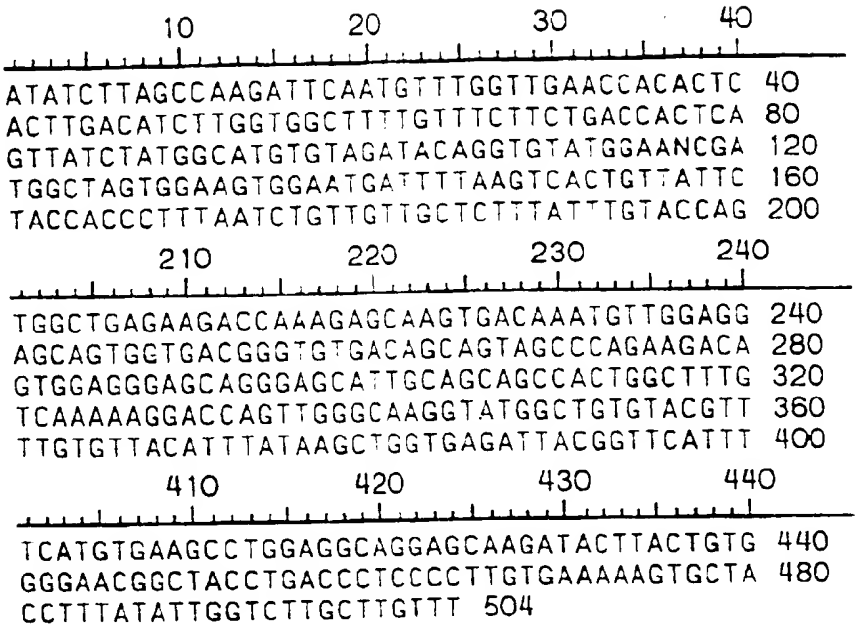

Figure 11 cont.

alpha-SYN exon 6

```
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CAATGTTTCCCCGGAGGCATTGTGGAGTTTAGAATGCCAG 80
TAGTAATATTAAGGTGTGCCATTTTCAAGATCCGTGGCCA 120
ACATCCCTATATGTAAGATTTTTCCAAAACATGGTTCTGA 160
TTTTTAAAAGTGAAAAATGCTACTTCATCATGTTCTTTTT 200
        210        220        230        240
GTGCTTCTTACTTTAAATATTAGAATGAAGAAGGAGCCCC 240
ACAGGAAGGAATTCTGGAAGATATGCCTGTGGATCCTGAC 280
AATGAGGCTTATGAAATGCCTTCTGAGGTAGGAGTCCAAG 320
CTGAATCTTTCTAACAAGACAGTACCAAAAACCTGTCATT 360
GTCACATTTCTCTTTCATTAGTGCTTAGTGAGAATCATTT 400
        410        420        430        440
GCTCTCTACATGCTCATTACGTGGACAACTTGCAAGTTAA 440
GAATAGTTTTTACATTTTTAAAGGGTCCTTAAAAAAAAAG 480
AGGAGGAGGAAGATGAAGAAGAGGAAGAAAGGATGTAAAA 520
GAAATCATATGTAGTCCACATAGCTTAATATACNTACTAC 560
TTGACCCTTTACAGGAAAAGCTTTACTAACCCCTGCATTA 600
        610        620        630        640
GAGAATATATTTTTTTGCAAAAACATTGATTGTAAATTTT 640
AGTGTAAAGTGGGGAGCCATTTCCTATCTCATTGGCTGTC 680
CAGTGCTGATGCGTAATTGAAACTTATACTAACAGTGTGT 720
GCTGTCT 727
```

alpha-SYN exon 7

```
        10         20         30         40
TTTTGATTTTTCTAATATTAGGAAGGGTATCAAGACTACG  40
AACCTGAAGCCTAAGAAATATCTTTGCTCCCAGTTTCTTG  80
AGATCTGCTGACAGATGTTCCATCCTGTACAAGTGCTCAG 120
TTCCAATGTGCCCAGTCATGACATTTCTCAAAGTTTTTAC 160
AGTGTATCTCGAAGTCTTCCATCAGCAGTGATTGAAGCAT 200
       210        220        230        240
CTGTACCTGCCCCCACTCAGCATTTCGGTGCTTCCCTTTC 240
ACTGAAGTGAATACATGGTAGCAGGGTCTTTGTGTGCTGT 280
GGATTTTGTGGCTTCAATCTACGATGTTAAAACAAATTAA 320
AAACACCTAAGTGACTACCACTTATTTCTAAATCCTCACT 360
ATTTTTTTGTTGCTGTTGTTCAGAAGTTGTTAGTGATTTG 400
       410        420        430        440
CTATCATATATTATNAGATTTTTAGGTGTCTTTTAATGAT 440
ACTGTCTAAGAATAATGACGTATTGTGAAATTTGTTAATA 480
TATATNATACTTAAAAATATGTGAGCATGAAACTATGCAC 520
CTATAATACTAAATATGAAATTTTACCATTTTGCGATGTG 560
TTTTATTCACTTGTGTTTGTATATNAATGGTGAGAATTAA 600
       610        620        630        640
AATAAAACGTTATCTCATTGCAAAAATATTTTATTTTTAT 640
CCCATCTCACTTTAATAATAAAAATCATGCTTATAAGCAA 680
CATGAATTAAGAACTGACACAAAGGACAAAAATATAAAGT 720
TATTAATAGCCATTTGAAGAAGGAGGAATTTTAGAAGAGG 760
TAGAGAAAATGGAACATTAACCCTACACTCGGAATTCCCT 800
       810        820        830        840
GAAGCAACACTGCCAGAAGTGTGTTTTGGTATGCACTGGT 840
TCCTTAAGTGGCTGTGATTAATTATTGAAAGTGGGGTGTT 880
GAAGACCCCAACTACTATTGTAGAGTGGTCTATTTCTCCC 920
TTCAATCCTGTCAATGTTTGCTTTACGTATTTTGGGGAAC 960
TGTTGTTTGATGTGTATGTGTTTATAATTGTTATACATTT 1000
      1010       1020       1030       1040
TTAATTGAGCCTTTTATTAACATATATTGTTATTTTTGTC 1040
TCGAAATAATTTTTTTAGTTAAAATCTATTTTGTCTGATAT 1080
TGGTGTGAATGCTGTACCTTTCTGACAATAAATAATATNC 1120
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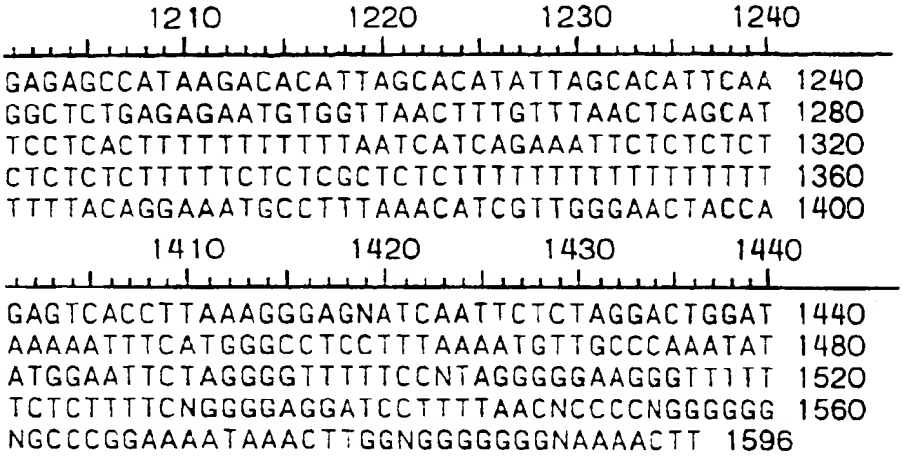
Figure 11 cont.

alpha-SYN exon 7



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/13071

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 C12N15/11 C07K16/18 A61K48/00
C12Q1/68 G01N33/68 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K C12Q G01N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHAPIRA A. H.: "Pathogenesis of Parkinson's disease." BAILLERES CLINICAL NEUROLOGY, vol. 6, no. 1, April 1997, pages 15-36, XP002083889	1-23, 57-61, 74
Y	see page 17, paragraph 2	24-56, 62-73
	see abstract	

Y	US 5 494 794 A (WALLACE DOUGLAS C) 27 February 1996 see the whole document	24-56, 62-73

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
10 November 1998	27/11/1998

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No
A	JAKES R. ET AL.: "Identification of two distinct synucleins from human brain." FEBS LETTERS, vol. 345, 1994, pages 27-32, XP002078475 cited in the application & UEDA K. ET AL.: "Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease." PROC. NATL. ACAD. SCI. USA, vol. 90, 1993, pages 11282-11286, see figure 2 ---	1-74
A	CHEN X. ET AL: "The human NACP/alpha-synuclein gene: chromosome assignment to 4q21.3-q22 and TaqI RFLP analysis." GENOMICS, vol. 26, no. 2, 1995, pages 425-427, XP002083890 cited in the application ---	1-74
A	POLYMEROPOULOS M. H. ET AL.: "Mapping of a gene for Parkinson's disease to chromosome 4q21-q23." SCIENCE, vol. 274, 1996, pages 1197-1199, XP002083891 cited in the application see the whole document ---	1-74
A	MAROTEAUX L. AND SCHELLER R. H.: "The rat brain synucleins; family of proteins transiently associated with neuronal membrane." MOLECULAR BRAIN RESEARCH, vol. 11, 1991, pages 335-343, XP002083892 cited in the application see figure 1 ---	1-74
P,X	NUSSBAUM R. L. AND POLYMEROPOULOS M. H.: "Genetics of Parkinson's disease." HUMAN MOLECULAR GENETICS, vol. 6, no. 10, 1997, pages 1687-1691, XP002083893 see the whole document ---	1-74
P,X	GOEDERT M.: "The awakening of alpha-synuclein." NATURE, vol. 388, 17 July 1997, pages 232-233, XP002083894 see the whole document --- -/--	1-74

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/13071

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
P,X	POLYMEROPOULOS M. H. ET AL.: "Mutation in the alpha-synuclein gene identified in families with Parkinson's disease." SCIENCE, vol. 276, 27 June 1997, pages 2045-2047, XP002083895 see the whole document	1-74

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. onal Application No
PCT/US 98/13071

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5494794 A	27-02-1996	WO 9409162 A	28-04-1994

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(74) Agents: SCHNELLER, John, W. et al.; Spencer & Frank, Suite 300 East, 1100 New York Avenue, N.W., Washington, DC 20005–3955 (US).

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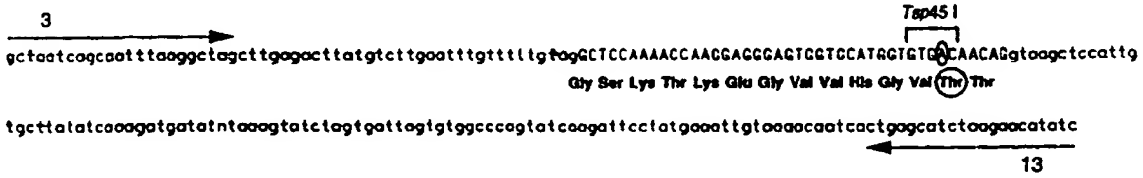

(57) Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2 percent. It was recently reported that a PD susceptibility gene is located on the long arm of human chromosome four. The present invention reports the subsequent identification of a mutation in the alpha synuclein gene, which codes for a presynaptic protein thought to be involved in neuronal plasticity. The finding of a specific molecular alteration which is causative for PD will permit the detailed understanding of the pathophysiology of the disorder, which will lead to potential therapeutic interventions, as well as a means for diagnosing individuals having an increased risk of developing the disease.

*(Referred to in PCT Gazette No 15/1999, Section II)

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CLONING OF A GENE MUTATION FOR PARKINSON'S DISEASE

BACKGROUND OF THE INVENTION

1. Field of the Invention

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2 percent. A pattern of familial aggregation has been documented for the disorder, and it was recently reported that a PD susceptibility gene in a large Italian kindred is located on the long arm of human chromosome 4. We have identified a mutation in the alpha synuclein gene, which codes for a presynaptic protein thought to be involved in neuronal plasticity, in the Italian kindred and in three unrelated families of Greek origin with autosomal dominant inheritance for the PD phenotype. This finding of a specific molecular alteration which is causative for PD will permit the detailed understanding of the pathophysiology of the disorder. In addition, methods of screening nucleic acids for the presence of mutations in the synuclein gene to test for predisposition to Parkinson's Disease are now possible.

2. Technology Background

Parkinson's disease (PD) was first described by James

Parkinson in 1817 (1). The clinical manifestations of this neurodegenerative disorder include resting tremor, muscular rigidity, bradykinesia and postural instability. A relatively specific pathological feature accompanying the neuronal degeneration is the intracytoplasmic inclusion body, known as the Lewy body, which is found in many regions including the substantia nigra, locus ceruleus, nucleus basalis, hypothalamus, cerebral cortex, cranial nerve motor nuclei, and the central and peripheral divisions of the autonomic nervous system (2).

In many cases a heritable factor predisposes to the development of the clinical syndrome (2). We have recently shown that genetic markers on human chromosome 4q21-q23 segregate with the PD phenotype in a large family of Italian descent (3). The clinical picture of the PD phenotype in the Italian kindred has been well documented to be typical for PD, including Lewy bodies, with the exception of a relatively earlier age of onset of illness at 46 ± 13 years. In this family the penetrance of the gene has been estimated to be 85%, suggesting that a single gene defect is sufficient to determine the PD phenotype.

We now report the identification of a mutation in the alpha synuclein gene that is associated with Parkinson's disease. The mutation, an Ala53Thr substitution, was found to be linked to the PD phenotype in four independent PD

families and absent from 314 control chromosomes, providing strong genetic evidence that this mutation in the human alpha synuclein gene is causative for the PD phenotype in these families.

The Ala53Thr substitution is localized in a region of the protein whose secondary structure predicts an alpha helical formation, bounded by beta sheets. Substitution of the alanine with threonine is predicted to disrupt the alpha helix and extend the beta sheet structure. Beta pleated sheets are thought to be involved in the self aggregation of proteins which could lead to the formation of amylcid like structures (6).

This was already tested in the case of NAC35, the 35 amino acid peptide derived from alpha-synuclein that was first isolated from plaques found in patients with Alzheimer's disease (4). NAC35 was shown to self aggregate and form amyloid fibril which shared the 'amyloid' characteristics of insolubility in aqueous solutions and green birefringence under polarized light, subsequent to Congo red staining (6). NAC35 is located in the middle of the alpha synuclein molecule and extends from amino acid 61 to amino acid 95. Residue 53, which is found to be mutated in PD, is outside the NAC35 peptide found in amyloid plaques. However, the true size of the NAC peptide involved in the plaques is not known since the protease used to

isolate the peptide from AD tissue cuts at lysine 60 of the alpha synuclein protein. It is therefore possible that amino acid 53 may be part of the NAC peptide found in plaques. In crosslinking experiments with beta amyloid (Abeta), it was demonstrated (6) that residues 1-56 and 57-97 specifically bind amyloid and that a synthetic peptide consisting of residues 32-57 performed similarly.

Three members of the synuclein family have been characterized in the rat, with SYN1 exhibiting 95% homology with the human alpha-synuclein protein (7). SYN 1 of the rat is expressed in many regions of the brain, with high levels found in the olfactory bulb and tract, the hippocampus, dentate gyrus, habenula, amygdala and piriform cortex, and with intermediate levels in the granular layer of the cerebellum, substantia nigra, caudate-putamen, and dorsal raphe (7). This pattern of expression coincides with the distribution of the Lewy bodies found in brains of patients with Parkinson's disease. It is also interesting to note that decrease in olfactory sense often accompanies the syndromic features of Parkinson's disease, and in many cases it is proposed that hyposmia is a prodromic sign of the illness (8).

In the zebra-finch the homologue to alpha synuclein, synelfin, is thought to be involved in the process of song learning, suggesting a role for synuclein perhaps in memory

and learning (9). In contrast to humans, rats have a threonine at residue 53 of their homologues to the human alpha synuclein gene (Figure 4). Similarly, the zebra-finch synelfin carries a threonine at amino acid 53, whereas both Bos taurus and Torpedo californica do not (10). There are no reports that suggest the presence of Lewy bodies in the brains of the rat or the zebra finch or a phenotype resembling that of PD. Lack of any phenotype could be explained by a combination of factors, including the following: the relative short life span of rodents may prohibit the observation of a late onset disorder, interaction with other cellular components not present in the rat may be required for the phenotype, absence of a critical environmental trigger in the rodents, or finally a heterozygous status Ala/Thr may be necessary for the production of a phenotype.

Studies of early onset AD have previously documented that missense mutations can cause an adult onset neurodegenerative disorder. Of the 31 mutations described so far in the loci for presenilin 1 and 2, thirty were missense and one was a splice variant (11). Missense mutations in the prion protein have also been implicated in the amyloid production seen in Gerstmann-Straussler-Scheinker and Creutzfeld-Jakob diseases, both forms of spongiform encephalopathy (12). Studies in these neurodegenerative

disorders have pointed to the importance of the physical chemical properties of mutant cellular proteins in initiating and propagating neuronal lesions leading to disease. Similar studies in the synuclein protein family may provide valuable insights into the etiology and pathogenesis of PD.

Similarly with the mutations in the presenilin genes in patients with early onset Alzheimer's disease, the mutation identified in the alpha synuclein gene is unlikely to account for the majority of sporadic and familial cases of PD. However, this mutation may account for a significant proportion of those families with a highly penetrant, early onset autosomal dominant PD phenotype.

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

3. Summary of the Invention

As described herein, we have discovered that particular mutations in the alpha synuclein gene are associated with predisposition to Parkinson's disease. Accordingly, the present invention includes an isolated nucleic acid comprising a mutated synuclein gene. In particular, the isolated nucleic acid of the present invention contains at

least one mutation in the alpha synuclein gene at base pair position 209 of Genbank # L08850, which, in particular, is a change from guanine to adenine. However, since other mutations in the alpha synuclein gene may also lead to Parkinson's Disease (PD), other mutations are also included. In addition, it is conceivable that mutations in the related beta (46)(SEQ ID NO 11) and gamma (SEQ ID NOs 12 and 13) synuclein genes may also lead to PD. Thus, mutated homologues of the alpha synuclein gene are also included in the present invention. Vectors comprising the isolated nucleic acid and host cells comprising such vectors are included as well.

Knowledge of particular genes that are associated with PD allows for the search for other specific PD mutations. Accordingly, the present invention also includes a method of using a synuclein gene sequence to identify specific PD mutations. Such mutations may occur in an unrelated population or in a family that demonstrates passage of PD within the family tree.

Since knowledge of mutations associated with Parkinson's disease allows the development of genetic screens that test for an individual's chances of being predisposed to the disease, and such tests may be performed by hybridization analysis using oligonucleotides complementary to the sequence of interest or by PCR amplification using oligonucleotides that are complementary to sequences flanking the mutation, the present invention also includes oligonucleotides

complementary to a portion of the synuclein gene, wherein said portion comprises or flanks a mutation associated with predisposition to Parkinson's Disease. In particular, the oligonucleotides of the present invention will have a sequence that is complementary to a sequence from the alpha synuclein gene that includes or flanks base pair position 209. And in particular, this mutation is a change from guanine to adenine at this position.

Vectors comprising an isolated nucleic acid encoding a mutated synuclein gene will allow the production and isolation of the mutant protein in an appropriate host cell using techniques well known in the art. Alternatively, peptides may be chemically synthesized using techniques also well known in the art. Isolation of such a protein or peptides thereof will allow the study of the molecular mechanisms which lead to development of Parkinson's disease. Accordingly, the present invention also includes an isolated synuclein protein or peptide containing at least one mutation. In particular, this mutation is at a position corresponding to the fifty-third amino acid in the native alpha synuclein protein, and in particular, this mutation is an alanine to threonine substitution.

Peptides corresponding to portions or the entirety of a synuclein gene may be useful as drugs for inhibiting the self-aggregation of mutant proteins that is thought to lead to Parkinson's disease. Accordingly, the present invention includes a method of testing peptides and other compounds for

the ability to interfere with this self-aggregation. Self-aggregation can be tested using a number of established methods, including Congo red staining, electron microscopy pictures of amyloid fibrils, and circular dichroism (CD) spectrophotometry. Using a peptide derived from the alpha synuclein protein that includes the mutant THR amino acid at position 53 alone or in combination with a normal peptide may allow testing for drugs that can inhibit the aggregation or dissolve an aggregate. This procedure can be used to rapidly identify agents that could be used in animal studies, clinical trials, or as diagnostic tools.

Possession of isolated synuclein proteins or peptides will also allow the isolation of specific antibodies using techniques well known in the art. Such antibodies may distinguish a mutant synuclein protein from its wildtype counterpart, and therefor could also be used in diagnostic screens. Alternatively, such antibodies may also be used to inhibit the self-aggregation of proteins during the progression of Parkinson's disease. Accordingly, the present invention also includes antibodies specific for a mutated synuclein protein or peptide. It should be understood that useful derivatives of such antibodies, such as Fv fragments and Fab fragments, are also included.

The above aspects of the present invention will allow methods of detecting subjects at increased risk for Parkinson's Disease. Such a method comprises obtaining a sample comprising nucleic acids from the subjects, and

detecting in the nucleic acids the presence of a mutation which is associated with Parkinson's disease. In particular, the mutation detected by the method of the present invention is located on human chromosome four, preferably in the alpha synuclein gene. In particular, the mutation causes an amino acid substitution at position 53 of the alpha synuclein gene, which is, in particular, an alanine to threonine substitution.

The detecting step of the method of the present invention may be accomplished several different ways as will be described in further depth below. All such methods are well known to those of ordinary skill in the art.

For instance, said detecting step may comprise combining a nucleotide probe which selectively hybridizes to a nucleic acid containing a mutation associated with a predisposition to Parkinson's disease, and detecting the presence of hybridization. Such a probe may be an oligonucleotide that is complementary to a portion of the synuclein gene, wherein said portion comprises the mutation. In particular, such an oligonucleotide is complementary to a mutated alpha synuclein gene having at least one mutation at base pair position 209. In particular, this mutation is a change from guanine to adenine.

The detecting step of the method of the present invention may also comprise amplifying a nucleic acid product comprising said mutation, and detecting the presence of said mutation in the amplified product using any nucleic acid

sequencing procedure known in the art. Alternatively, the detecting step may comprise selectively amplifying a nucleic acid product comprising said mutation, and detecting the presence of amplification using any appropriate method known in the art. Such methods include gel electrophoresis of amplified nucleic acids, and detection of radiolabeled amplified nucleic acids using autoradiographic film or any other detection method known in the art.

The amplifying step of the present invention may be performed using the polymerase chain reaction (PCR), reverse transcriptase PCR (RTPCR), or any other type of PCR reaction known in the art. Accordingly, such a step will comprise at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids. In particular, said amplifying step uses two oligonucleotides. And in particular, the two oligonucleotides have the sequences given in SEQ ID NOs 2 and 3.

Alternatively, the detecting step of the method of the present invention comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of a nucleic acid sample. Such a detecting means will be possible when a mutation associated with a predisposition to Parkinson's disease results in a sequence having a new restriction endonuclease cleavage site, or loss of a native restriction endonuclease site. In particular, the mutation associated with Parkinson's disease results in the formation of a non-native *Tsp*45I restriction endonuclease site.

Alternatively, the detecting step of the present invention may be performed using a gene-specific primer and subsequent chain termination at the position of the mutation using DNA polymerase and labeled nucleotides or dideoxynucleotides. The presence of nucleic acids in which a dideoxynucleotide corresponding to the mutation of interest is incorporated at the appropriate position may be detected by any means known in the art, including detection of radiolabeled dideoxynucleotides using, for example, autoradiographic film, or detection of fluorescently-labeled dideoxynucleotides.

Since the methods and compounds of the present invention will be useful in diagnostic screening procedures aimed at identifying individuals having a predisposition for Parkinson's disease, the present invention also includes diagnostic kits which include the compounds of the present invention in a form that allows such compounds to be used quickly and easily for the designated purpose.

Finally, the inventors also contemplate that the isolated nucleic acid, oligonucleotides and antibodies of the present invention may eventually be used in methods directed at the correction or suppression of Parkinson's disease. For example, oligonucleotides or expression vectors designed from the synuclein nucleic acid sequences of the present invention may one day be used in antisense therapy directed at inhibiting expression of the mutated synuclein protein in

patients with Parkinson's disease, or in individuals having a predisposition for Parkinson's disease. Similarly, antibodies specific for the mutated synuclein protein may be useful in therapies directed at inhibiting the self-aggregation of mutated proteins or peptides in patients having Parkinson's disease. Knowledge of gene(s) associated with the development of Parkinson's disease may also allow the design of transgenic animals which express the mutant gene(s). Such animals may serve as a useful disease model, allowing one to test the effects of candidate therapies and therapeutic compositions in the treatment or inhibition of Parkinson's disease.

A detailed description of the present invention is now provided, and should not be considered as limiting on the present invention as described above.

4. Brief Description of the Drawings

Figure 1.

DNA sequence of the PCR product used for mutation detection (SEQ ID NO 1). Oligonucleotide primers are shown by arrows and the numerals 5 and 13 (SEQ ID NO 2 and 3). Intron sequence is shown in lower case and exon sequence in upper case. Amino acid translation of the exon is shown below the DNA sequence. The circled base represents the G209A change in the mutant allele. The resulting amino acid

Ala53Thr change is represented by the circled amino acid. The newly created *Tsp*45 I site is indicated above the DNA sequence.

Figure 2.

Mutation analysis of the G209A change is shown in a subpedigree of the Italian kindred. Filled symbols represent affected individuals. Numerical identifiers, denote the individuals immediately above. *Tsp*45 I digestion of PCR products is shown at the bottom of the figure, and fragment sizes are indicated on the right in base pairs.

Figure 3.

Mutation analysis of the G209A change in RT PCR products (7). Lane 1: 100 bp ladder, lanes 2 and 3 normal control, lanes 4 and 5 PD patient, lane 6 negative control without RT enzyme. Sizes are indicated on the right in base pairs. Lanes 2 and 4 show uncut DNA and lanes 3 and 5 show DNA cut with *Tsp*45 I.

Figure 4.

Sequence alignments of alpha synuclein homologues in different species. Accession numbers for the sequences used were as follows: Homo sapiens Swiss-Prot P37840 (SEQ ID NO 4), Rattus norvegicus Swiss-Prot P37377 (SEQ ID NO 5), Bos

taurus Swiss-Prot P33567 (SEQ ID NO 6), Serinus canaria genbank L33860 (SEQ ID NO 7), Torpedo californica Swiss-Prot P37379 (SEQ ID NO 8). Numbering on top of the alignments is according to the human sequence. Amino acid 53, which is the site of the Ala53Thr change, is circled.

Figure 5.

The pedigree of a large family with PD (3). The clinical and pathological features of some members of this kindred were previously reported.

Figure 6.

Multipoint LOD score analysis between chromosome 4q markers and the PD locus.

Figure 7.

A table of human synuclein clones identified from various databases. Columns labeled 5' and 3' show the sequence acquisition numbers. Clones were identified by homology to protein or nucleic acid sequence. Human gamma clones were identified by homology to known mouse and rat gamma synuclein sequences.

Figure 8.

Sequence of BAC clone 139A20 for human beta synuclein.

BAC clone was isolated using primers to known database sequences described in Figure 7. The sequence shown includes all coding exon sequences and some non-coding intronic sequences. (SEQ ID NO:11)

Figure 9.

Sequence from the 5' end of BAC clone 174P13 for human gamma synuclein. The BAC clone was isolated with primers from the database sequences described in Figure 7. (SEQ ID NO:12)

Figure 10.

Sequence from the 3' end of BAC clone 174P13 for human gamma synuclein. BAC clone was isolated as described in Figure 9. The entire human gamma synuclein gene has now been sequenced and has been deposited in GenBank: accession number AF044311. (SEQ ID NO: 13)

Figure 11.

Sequence of exons 1-7 of the human alpha synuclein gene, plus some flanking intronic sequence for each exon. (SEQ ID NOs 14-19)

5. Detailed Description of the Invention

Definitions

Unless defined otherwise, all technical and scientific

terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are defined below.

This invention provides a method of diagnosing or predicting a predispcsition to Parkinson's disease. The method comprises detecting in a sample from a subject the presence of a mutation, for example, in nucleotide position 209 of the human alpha synuclein gene. The presence of the mutation indicates the presence of or a predisposition to Parkinson's disease.

As used herein, the term "gene" primarily relates to a coding sequence, but can also include some or all of the surrounding or flanking regulatory regions or introns. The term "gene" specifically includes artificial or recombinant genes created from cDNA or genomic DNA, including recombinant genes based upon splice variants.

As used herein, the term "synuclein" gene or protein may refer to the alpha synuclein gene or any homologue thereof. A "homologue" is understood to mean any related gene or protein that is at least 25% homologous to the alpha synuclein gene or protein or performs a related function. Preferably, a synuclein gene or protein refers to alpha, beta or gamma synuclein, but most preferably refers to alpha

synuclein.

As used herein, an "isolated nucleic acid" is a ribonucleic acid, deoxyribonucleic acid, or nucleic acid analog comprising a polynucleotide sequence that has been isolated or separated from sequences that are immediately contiguous, i.e. on the 5' and 3' ends, in the naturally occurring genome of the organism from which it is derived. The term therefor includes, for example, a recombinant nucleic acid which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule independent from any other sequences.

An isolated nucleic acid of the present invention may be "operatively linked" to an expression control sequence or regulatory region. As used herein, "operatively linked" means that the components are joined in such a way that the expression, transcription or translation of the sequence is under the influence or control of the regulatory region.

As used herein, a "predisposition" to Parkinson's disease means an increased probability of developing Parkinson's disease during the subject's lifetime as compared to the average individual.

Pertaining to this probability, a LOD score is a measure of genetic linkage used herein, defined as the log ratio of the probability that the data would have arisen if the loci are linked to the probability that the data could have arisen from unlinked loci. The conventional threshold for declaring

linkage is a LOD score of 3.0, that is, a 1000:1 ratio (which must be compared with the 50:1 probability that any random pair of loci will be unlinked).

As used herein, reference to "base pair position" or "amino acid position" when referring to an isolated nucleic acid, probe, protein or peptide always indicates the relative position in the native gene or protein.

A "probe" refers to a nucleic acid which has sufficient nucleotides surrounding the codons at the mutation positions to distinguish the nucleic acid from nucleic acids encoding non-related genes. The specific length of the nucleic acid is a matter of routine choice based on the desired function of the sequence. For example, if one is making probes to detect the mutation in base pair position 209, the length of the nucleic acid is preferably small, but must be long enough to prevent hybridization to undesired background sequences. However, if the desired hybridization is to a nucleic acid which has been amplified, background hybridization is less of a concern and a smaller probe can be used. In general, such a probe will be between 10 and 100 nucleotides, especially between 10 and 40 and preferably between 15 and 25 nucleotides in length. It is apparent to one of skill in the art that nucleotide substitutions, deletions, and additions may be incorporated into the polynucleotides of the invention. However, such nucleotide substitutions, deletions, and additions should not substantially disrupt the ability of the polynucleotide to hybridize under conditions that are

sufficiently stringent to result in specific hybridization.

As used herein with respect to genes, "the term "normal" refers to a gene which encodes a normal protein. As used herein with respect to proteins, the term "normal" means a protein which performs its usual or normal physiological role and which is not associated with, or causative of, a pathogenic condition or state. Therefor, the term "normal" is generally synonomous with the phrase "wild type".

For any given gene or corresponding protein, a multiplicity of normal allelic variants may exist, none of which is associated with the development of a pathogenic condition or disease state. Such normal allelic variants include, but are not limited to, variants in which one or more nucleotide substitutions do not result in a change in the encoded amino acid sequence.

As used herein, the term "mutation" generally refers to a mutation in a gene that is associated with a predisposition to Parkinson's disease. "Mutant" can specifically refer to a mutation at nucleotide position 209 of the synuclein gene, and is in particularly a G to A transition. However, other mutations in the synuclein gene or other genes which are associated with a predisposition to Parkinson's disease are also encompassed. Furthermore, the term "mutation" is not limited to transition mutations, but can also mean a deletion, insertion or transversion as well.

The term "mutant", as it applies to synuclein genes, is not intended to embrace sequence variants which, due to the

degeneracy of the genetic code, encode proteins identical to the normal sequences disclosed or otherwise enabled herein; nor is it intended to embrace sequence variants which, although they encode different proteins, encode proteins which are functionally equivalent to normal synuclein proteins. The term "mutant" means a protein which does not perform its usual or normal physiological role and which is associated with, or causative of, a pathogenic condition or state.

Since a mutation can be a substitution, deletion or insertion, a mutated synuclein "protein" is understood to refer to the amino acid sequence resulting from any such mutation whether the resulting protein is shorter, longer or modified, i.e. due to an alteration in reading frame or generation of stop codon. In addition, "peptide" is understood to refer to a portion of the mutated protein that is preferably at least five base pairs long, and more preferably at least 10 base pairs long. This portion may be derived from the amino or carboxyl terminus, or it may be an internal portion of the full length protein. As such, a peptide may be chemically synthesized using any method known in the art, or may be made using a recombinant DNA technology and an appropriate purification scheme or isolated from the native protein using enzymatic digestion.

As used herein, the term "substantially pure" means a preparation which is at least 60% by weight the compound of interest. Preferably the preparation is at least 75%, more

preferably 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate method, i.e. column chromotography, gel electrophoresis or HPLC analysis.

"Specific or selective hybridization" as used herein means the formation of hybrids between a probe nucleic acid (e.g., a nucleic acid which may include substitutions, deletions, and/or additions) and a specific target nucleic acid (e.g., a nucleic acid having the mutated sequence), wherein the probe preferentially hybridizes to the specific target such that, for example, a band corresponding to the mutated DNA or restriction fragment thereof can be identified on a Southern blot, whereas a corresponding normal or wild-type DNA is not identified or can be discriminated from a variant DNA on the basis of signal intensity. Hybridization probes capable of specific hybridization to detect a single-base mismatch may be designed according to methods known in the art (13-17).

"Stringent" as it refers to hybridization conditions is a term of art understood by those of ordinary skill to refer to those conditions of temperature, chaotrophic acids, buffer and ionic strength which permit hybridization of a particular nucleic acid sequence to its complementary sequence and not to substantially different sequences. The exact conditions which constitute "stringent" conditions depend on the nature of the nucleic acid sequence, the length of the sequence, and the frequency of occurrence of subsets of that sequence

within other non-identical sequences. By varying hybridization conditions from a level of stringency at which non-specific hybridization occurs to a level at which only specific hybridization occurs, one of ordinary skill in the art can, without undue experimentation, determine conditions which will allow a given sequence to hybridize only with complementary sequences.

Suitable ranges of stringency conditions are described in Sambrook et al. (13). Hybridization conditions, depending on the length and commonality of a sequence, may include temperatures of 20°C-65°C and ionic strengths from 5X to 0.1X SSC. Highly stringent hybridization conditions may include temperatures as low as 40°C-42°C (when denaturants such as formamide are included) or up to 60°C-65°C in ionic strengths as low as 0.1X SSC. These ranges are, however, only illustrative and, depending on the nature of the target sequence, and possible future technological developments, may be more stringent than necessary. Appropriate conditions may be determined for each specific nucleic acid sequence or oligonucleotide probe using standard control and a level of experimentation that is not considered to be undue by those of skill in the art.

As discussed below in greater detail, the mutation can be detected by many methods. For example, the detecting step

can comprise combining a nucleotide probe capable of selectively hybridizing to a nucleic acid containing the mutation with a nucleic acid in the sample and detecting the presence of hybridization. Additionally, the detecting step can comprise amplifying the nucleotides surrounding and including the mutation and detecting the presence of the mutation in the amplified product, or selectively amplifying the nucleotides of the mutation and detecting the presence of amplification. Finally, the detecting step can comprise detecting the presence or absence of a restriction fragment created by an enzyme digest of the sample nucleic acid, or any other detection means known in the art.

Detection Techniques

Once the location of a PD-relevant mutation is known, the methods to detect such a mutation are standard in the art. The sequence of various nucleotide probes can be determined from the known sequence of the relevant gene, especially the sequences surrounding the mutation.

Detection of point mutations using direct probing involves the use of oligonucleotide probes which may be prepared, for example, synthetically or by nick translation. The probes may be suitably labeled using, for example, a radio label, enzyme label, fluorescent label, biotin-avidin label and the like for subsequent visualization by any appropriate assay, i.e. Southern blot hybridization. In this procedure, the labeled probe is reacted with sample DNA that is bound, for example,

to a nylon filter under conditions such that only fully complementary sequences hybridize. The areas that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling may then be visualized, for example, by autoradiography.

Methods of manipulating hybridization conditions to achieve varying degrees of specificity are well known in the art. For example, tetra-alkyl ammonium salts may be used to bind selectively to A-T base pairs, thus displacing the dissociation equilibrium and raising the melting temperature. At 3M Me 4NCl, this is sufficient to shift the melting temperature to that of G-C pairs. This results in a marked sharpening of the melting profile. The stringency of hybridization in such an experiment is usually 5°C below the Ti (the irreversible melting temperature of the hybrid formed between the probe and its target sequence) for the given chain length. For a 20mer oligonuclectide probe, the recommended hybridization temperature is about 58°C. The washing temperatures are unique to the sequence under investigation and need to be optimized for each variant.

There are certainly other ways known in the art for adjusting hybridization conditions in view of desired specificity. For instance, although hybridization may be carried out in accordance with conventional hybridization methods under suitable conditions with respect to e.g. stringency, incubation time, temperature, etc, the choice of

conditions will depend on the desired degree of complementarity between the fragments to be hybridized. A high degree of complementarity requires more stringent conditions such as low salt concentrations, low ionic strength of the buffer and higher temperatures, whereas a low degree of complementarity requires less stringent conditions, e.g. higher salt concentration, higher ionic strength of the buffer or lower temperatures, for the hybridization to take place.

The support to which DNA or RNA fragments of the sample to be analyzed are bound in denatured form is preferably a solid support and may have any convenient shape. Thus, it may, for instance, be in the form of a plate, e.g. a thin layer or a microtiter plate, a strip, a solid particle e.g. in the form of a bead such as a latex bead, a filter, a film or paper. The solid support may be composed of a polymer, preferably nylon or nitrocellulose.

Alternative probing techniques, such as ligase chain reaction (LCR), may involve the use of mismatch probes, i.e., probes which are fully complementary with the target except at the point of the mutation. The target sequence is then allowed to hybridize both with oligonucleotides which are fully complementary and have oligonucleotides containing a mismatch, under conditions which will distinguish between the two. By manipulating the reaction conditions according to the above considerations, it is possible to obtain hybridization only where there is full complementarity. If a mismatch is

present there is significantly reduced hybridization.

The polymerase chain reaction (PCR) is a technique that amplifies specific DNA sequences with remarkable efficiency. Repeated cycles of denaturation, primer annealing and extension carried out with Taq polymerase, e.g., a heat stable DNA polymerase, leads to exponential increases in the concentration of desired DNA sequences. Given a knowledge of the nuclectide sequence of the mutations, synthetic oligonucleotides can be prepared which are complementary to sequences which flank the DNA of interest. Each oligonucleotide is complementary to one of the two strands. The DNA is denatured at high temperatures (e.g., 95°C) and then reannealed in the presence of a large molar excess of oligonuclectides. The oligonucleotides, oriented with their 3' ends pointing towards each other, hybridize to opposite strands of the target sequence and prime enzymatic extension along the nucleic acid template in the presence of the four deoxyribonucleotide triphosphates. The end product is then denatured again for another cycle. After this three-step cycle has been repeated several times, amplification of a DNA segment by more than one million-fold can be achieved. The resulting DNA may then be directly sequenced in order to locate any genetic alteration. Alternatively, it may be possible to prepare oligonuclectides that will only bind to altered DNA, so that PCR will only result in multiplication of the DNA if the mutation is present. Following PCR, direct visualization or allele-specific oligonucleotide

hybridization (18) may be used to detect the Parkinson's disease point mutation. Alternatively, PCR may be followed by restriction endonuclease digestion with subsequent analysis of the resultant products.

As shown in the examples, the substitution of G for A at base pair 209 of the synuclein, results in the gain of a *Tsp*45I site. The gain of this restriction endonuclease recognition site facilitates the detection of the Parkinson's disease mutation using restriction fragment length polymorphism (RFLP) analysis or by detection of the presence or absence of the restriction site in a PCR product that spans base pair position 209.

For RFLP analysis, DNA is obtained, for example from the blood cells of the subject suspected of having Parkinson's disease and from a normal subject, is digested with a restriction endonuclease, and subsequently separated on the basis of size by agarose gel electrophoresis. The Southern technique can then be used to detect, by hybridization with labeled probes, the products of endonuclease digestion. The patterns obtained from the Southern blot can then be compared. Using such an approach, an additional restriction endonuclease site, such as a *Tsp*45I site, is detected by determining the number of bands detected and comparing this number to the normal subject.

The creation of a new restriction site as a result of a nucleotide substitution at a disclosed mutation site can be

readily determined by reference to the genetic code and a list of nucleotide sequences recognized by restriction endonucleases (19).

In general, primers for PCR are usually about 20 bp in length, and are most preferably 15-25 bp. Denaturation of strands usually takes place at 94°C. and extension from the primers is usually at 72°C. The annealing temperature varies according to the sequence under investigation. Examples of reaction times are: 20 mins denaturing; 35 cycles of 2 min, 1 min, 1 min for annealing, extension and denaturation; and finally a 5 min extension step.

PCR "amplification of specific alleles" (PASA) may also be used to detect the presence of the PD mutation. PASA is a rapid method of detecting single-base mutations or polymorphisms (22-28). PASA (also known as allele specific amplification) involves amplification with two oligonucleotide primers such that one is allele-specific. The desired allele is efficiently amplified, while the other allele(s) is poorly amplified because it mismatches with a base at or near the 3' end of the allele-specific primer. Thus, PASA or the related method of PAMSA may be used to specifically amplify the mutation sequences of the invention. Where such amplification is done on genetic material (or RNA) obtained from an individual, it can serve as a method of detecting the presence of the mutations.

As mentioned above, a method known as ligase chain reaction (LCR) can be used to successfully detect a

single-base substitution (29, 30). LCR probes may be combined or multiplexed for simultaneously screening for multiple different mutations. Thus, LCR can be particularly useful where multiple mutations are predictive of the same disease.

Finally, the Parkinson's disease mutation of the present invention may also be detected using chain termination with labeled dideoxynucleotides. For instance, U.S. Patent No. 5,047,519 to Hobbs et al. discloses fluorescently-labeled nuclectides as chain-terminating substrates for a fluorescence-based DNA sequencing method. With such substrates and knowledge of the gene sequence of interest, it is possible to design an assay using a gene-specific primer to initiate a polymerase reaction immediately flanking the position of the mutation, employing color-coded dideoxynucleotide terminators such that the specific nucleotide at the position of the mutation may be easily determined via a colorimetric assay.

Transgenic Animals and Cell Lines

Having identified subjects having a predisposition to Parkinson's disease associated with a specific mutation, the subjects can participate in the screening of putative agents capable of treating Parkinson's disease. This method comprises administering the test agent to the subject, which may be a human, which has a mutation in a gene associated with Parkinson's disease and monitoring the effect of the

agent on the subject's condition. If the symptoms of Parkinson's disease improve, the agent can be used as a treatment for the disease.

In addition, it is possible to develop transgenic model systems and/or cell lines containing the mutated nucleic acid(s) for use, for example, as model systems for screening for drugs and evaluating drug efficiency. Additionally, such model systems provide a tool for defining the underlying biochemistry of, for instance, the mutated synuclein gene, thereby providing a rationale for drug design.

One approach to creating transgenic animals is to mutate the animal gene of interest by in vivo mutagenesis, transfer the mutant gene into embryonic stem cells by DNA transfection and inject the embryonic stem cells into blastocysts in order to retrieve offspring which carry the disease-causing mutation (31). Alternatively, the technique of microinjection of the mutated gene, into a one-cell embryo followed by incubation in a foster mother can be used. Alternatively, viral vectors, e.g., Adeno-associated virus, can be used to deliver the mutated gene to a stem cell, or may be used to target specific cells of a fully developed animal (32,33).

Antibodies and Recombinant Expression of Polypeptides

When the mutated gene product is a polypeptide, e.g. the 209 mutation, it can be used to prepare antisera and monoclonal antibodies using, for example, the method of Kohler and Milstein (34). Such monoclonal antibodies could

then form the basis of a diagnostic test, or may even be useful in therapies directed toward inhibiting the action of the mutant protein in a patient with Parkinson's disease.

Mutant polypeptides can also be used to immunize an animal for the production of polyclonal antiserum (35). For example, a recombinantly produced fragment of a variant polypeptide can be injected into a mouse along with an adjuvant so as to generate an immune response. Murine immunoglobulins which specifically bind the recombinant fragment can be harvested from the immunized mouse as an antiserum, and may be further purified by affinity chromatography or other means. Additionally, spleen cells are harvested from the mouse and fused to myeloma cells to produce a bank of antibody-secreting hybridoma cells, which can then be screened for clones that secrete immunoglobulins which bind the recombinantly produced fragment with an increased affinity. More specifically, immunoglobulins that selectively bind to the variant polypeptides but poorly or not at all to wild-type polypeptides are selected, either by pre-absorption with wild-type proteins or by screening of hybridoma cell lines for specific idiotypes that bind the variant but not wild-type.

These antibodies can be used to screen protein and tissue samples for the presence of mutated proteins. A colored enzymatic reaction occurs when the specific antibody remains bound to its target protein, in situ, after thorough washing, as directed by established protocols.

Gene expression

The nucleic acid sequences of the present invention will be capable of expressing the desired mutant or normal polypeptides in an appropriate host cell. For expression in host cells, the DNA sequences of the present invention will be operably linked to, i.e., positioned to ensure the functioning of, an expression control sequence. For example, such polynucleotides can include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site, and, optionally, an enhancer for use in eukaryotic expression hosts. In addition, the DNA sequence of the present invention may also be fused such that the reading frame is conserved to an appropriate signal sequence to facilitate export of the encoded protein across the cell membrane.

Expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. A variety of suitable expression vectors are disclosed in Sambrock et al. (13). Commonly, expression vectors will contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired DNA sequences.

E. coli is one prokaryotic host that is particularly useful for cloning and expression of the DNA sequences of the present invention because of the wide variety of available expression systems. Vectors suitable for use in E. coli are

known and are commercially available, i.e. pBR322 (13), pBLUESCRIPT (Stratagene), etc. Also, a variety of different types of expression systems may be used, including plasmids, cosmids, bacteriophage lambda, etc. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilus, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. Expression vectors for use in prokaryotic host cells will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any of a variety of well-known promoters may be used, such as the lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. A promoter may optionally contain an operator sequence for regulatable gene expression, and will have a ribosome binding site sequence for the initiation of translation.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (36). Vectors for use in eukaryotic cells are known and commercially available, i.e. pcDNA3 (Invitrogen). Eukaryotic cells are actually preferred, and a number of suitable host cell lines capable of secreting intact human proteins have been developed in the art, including CHO cells, COS cells, HeLa cells, myeloma cell lines, Jurkat cells, etc. Promoters for use in eukaryotic vectors may be cell-specific, or capable of being expressed

in a wide variety of cells, i.e. viral promoters.

Expression vectors of the present invention (e.g., comprising nucleic acid sequences encoding a mutant or normal polypeptide) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts.

Kits

The method lends itself readily to the formulation of test kits which can be utilized in diagnosis. Such a kit would comprise a carrier compartmentalized to receive in close confinement one or more containers wherein a first container may contain suitably labeled DNA probes. Other containers may contain reagents useful in the localization of the labeled probes, such as enzyme substrates. Still other containers may contain restriction enzymes (such as *Tsp*45I), buffers, etc., together with instructions for use.

DESCRIPTION OF THE INVENTION

Detailed Description of the Preferred Embodiments

The following laboratory procedures were used:

DNA samples were collected upon informed consent. High molecular weight genomic DNA was isolated from whole-blood

lysate by methods previously described (38). Genotyping was performed as previously described (39). Pairwise linkage analysis was performed using the MLINK program of the FASTLINK package (40-42). Allele frequencies were used as reported in the Genomic Data Base (http://gdbwww.gdb.org) and the Cooperative Human Linkage Consortium (CHLC) database (http://www.chlc.org). Multipoint analysis was performed using the LINKMAP program of the FASTLINK package. For the multipoint analysis allele frequencies were set to 1/n where n equals the number of alleles observed. In the two point analysis LOD scores were calculated for both the reported and the 1/n allele frequencies with minimal effect on the maximum LOD score observed. Simulations of multipoint analysis in a subset of the pedigree with different allele frequencies similarly indicated no significant effect on the scores calculated. Maximum LOD scores as shown were observed for the heterozygote and homozygote disease allele penetrance set to 0.99, which is similar to the PD allele penetrance previously reported ranging from 0.88 to 0.94 (3). All unaffected individuals used in the study were of age above the mean for onset of illness. Disease allele frequency was set to 0.0001. Distances and order of genetic markers were set as reported in the CHLC database. Overlapping three point analysis was performed for markers *D4S2361*, *D4S1647*, *D4S421* and the PD locus. The 12 allele *D4S2380* locus was not included because of prohibitive time run. Multipoint

analysis was performed on an IBM SP2 parallel computer and the SGI Challenge machine.

For mutation analysis genomic DNA was amplified with oligonucleotides (3): 5' GCTAATCAGCAATTTAAGGCTAG 3' (SEQ ID NO 2) and (13): 5' GATATGTTCTTAGATGCTCAG 3' (SEQ ID NO 3) of genbank ID: U46898, under standard PCR conditions. Sequence analysis was performed using the Perkin Elmer dye terminator cycle sequencing kit on an ABI 373 fluorescent sequencer (ABI, Foster City, CA). Restriction digestion was performed following the PCR with *Tsp*45 I according to manufacturer's protocol (New England Biolabs, Beverly, MA). The digested PCR products were electrophoresed on a 6% Visigel (Stratagene, La Jolla, CA), and visualized by ethidium bromide staining. Pedigree structure in Figure 2 has been slightly modified in order to protect patient confidentiality.

Total RNA was extracted from the lymphoblastoid cell line of an affected individual and first strand synthesis was performed by oligo dT priming (Gibco BRL, Gaithersburg, MD). Primers (1F) 5' ACGACAGTGTGGTGTAAAGG 3' (SEQ ID NO 9) and (13R) 5' AACATCTGTCAGCAGATCTC 3' (SEQ ID NO 10) corresponding to nucleotides 21-40 and 520-501 of genbank L08850 were used to amplify a product of 500 bp containing the mutation at nucleotide 209. PCR products were subjected to restriction digestion by *Tsp*45 I. The mutation at nt 209 creates a novel *Tsp*45 I site (Figure 1), so that the normal allele will be

restricted in 4 fragments of 249, 218, 24 and 9 bp, where the mutant allele will have 5 fragments of 249, 185, 33, 24 and 9 bp of size, as shown in Figure 3. Size standards used, were the 100 bp ladder (Gibco BRL, Gaithersburg, MD).

Example 1

In an effort to identify a genetic locus responsible for Parkinson's disease, we performed a genome scan in a large kindred of Italian descent with pathologically confirmed PD (Figure 5). The kindred originated in the town of Contursi in the Salerno province of Southern Italy (3). Some members emigrated to the United States, Germany and other countries. The extended family pedigree consists of 592 members with 60 individuals affected by PD. The average age of onset for the illness in this pedigree (Figure 5) has been shown to be 46 ± 13 years. One hundred and fourty genetic markers were typed in this pedigree at an average spacing of about 20 cm. Genetic markers at the cytogenetic location *4q21-q23* were the only ones to show linkage to the disease phenotype with a Zmax=6.00 at theta=0.00 for marker *D4S2380I* (see Table 1).

Table 1. Two point LOD scores between chromosome 4q markers and the PD locus

Locus	Two-point LOD scores at recombination fractions of: 0.00	0.01	0.05	0.10	0.20	0.30	0.40	Z_{max}	θ_{max}
D4S2361	-5.60	-0.83	0.30	0.54	0.43	0.21	0.06	0.55	0.12
D4S2380	6.00	5.90	5.30	4.60	3.00	1.50	0.50	6.00	0.00
D4S1647	5.22	5.07	4.47	3.71	2.26	1.05	0.30	5.22	0.00
D4S421	-2.42	0.45	0.77	0.65	0.38	0.22	0.09	0.77	0.05

Recombinations between the disease phenotype and genetic markers were observed in the proximal region for marker *D4S2361* and in the distal region for marker *D4S421*. Genetic markers *D4S2380* and *D4S1647* showed no obligate recombination events in the affected individuals.

Multipoint LOD score analysis between markers *D4S2361*-13cM-*D4S1647*-3cM-*D4S421* and the disease locus places the PD gene between markers *D4S2361* and *D4S421* at a recombination distance of 0.00 cM from marker *D4S1647* with a Zmax=6.04 (Figure 6). This location is favored from the alternative genetic intervals by a difference in the LOD score of greater than three LOD units.

Although expansions of unstable trinucleotide repeats are found in a number of human neurogenerative conditions, there is no evidence for an association of a CAG trinucleotide repeat expansion in families with PD (43). In

addition, genetic linkage studies in other families with PD-like illnesses do not support the involvement of several candidate genes (glutathione peroxidase, tyrosine hydroxylase, brain-derived neurotrophic factor, catalase, amyloid precursor protein, CuZn superoxide dismutase and debrisoquinone 4-hydroxylase) in the etiology of the disorder (44). Genes previously mapped in the general region of linkage include the loci for alcohol dehydrogenase, formaldehyde dehydrogenase, synuclein, UDP-N-acetylglycosamine phosphotransferase and others.

Our localization of a PD susceptibility gene represents the first genetic locus linked to PD. Other distinct clinicopathological entities associated with parksonian features are probably linked to other genetic loci. For example, Wilhelmsen-Lynch disease (disinhibition-dementia-parkinsonian-amyotrophy complex) is linked to the *17q21-q22* chromosomal region (45). If the pathogenesis of diseases affecting the nigrostriatal pathway includes environmental influences, then a range of mutations affecting vulnerable sites in the electron transport chain or enzyme polymorphisms influencing neurotoxin metabolism may vary the penetrance of PD by altering an individual's resistance to exogenous or endogenous agents. However, our finding of a highly penetrant genetic locus linked to PD suggested that abnormalities of a single gene may be sufficient to cause Parkinson's disease.

Example 2

In an effort to identify a specific gene between markers *D4S2361* and *D4S421* that is associated with predisposition to Parkinson's disease, we conducted sequence analysis of candidate genes in this region.

Alpha synuclein, a presynaptic nerve terminal protein, was originally identified as the precursor protein for the NAC peptide, a non beta amyloid component of Alzheimer's disease (AD) amyloid plaques (4). The human alpha synuclein gene was previously mapped in the 4q21-q22 region (5). We refined the mapping, and determined that the alpha synuclein gene is located within the non-excluded region harboring the PD gene in the Italian kindred. Thus alpha synuclein represented an excellent candidate locus for PD.

Sequence analysis of the fourth exon of the alpha synuclein gene revealed a single base pair G209A change from the published sequence of the gene (GenBank ID L08850), which results in an Ala53Thr substitution and the creation of a novel *Tsp*45 I restriction site (Figure 1). Mutation analysis for the G209A change in the Italian kindred shows complete segregation with the PD phenotype with exception of individual 30 (Figure 3), who is affected but not carrying this mutation. This individual apparently inherited a different PD mutation from his father, as we have shown that

he shares a genetic haplotype with his unaffected maternal uncle, individual 3, for genetic markers in the PD linkage region.

The frequency of this variation was studied in two general population samples, one consisting of 120 chromosomes of the parents of the CEPH reference families, and the other consisting of 194 chromosomes of unrelated individuals from the blood bank in Salerno, Italy, a city near the town from which the family originated. Of these 314 general population chromosomes none was found to carry the G209A mutation. Fifty two patients of Italian descent with sporadic PD were also screened for the mutation (Figure 2), along with 5 probands from previously unpublished Greek families with PD. The Ala53Thr change was found to be present in three of the Greek kindreds and it segregated with the PD phenotype. In those three Greek kindreds it is worth noting that the age of onset for the disease is relatively early, ranging from the mid 30's to the mid 50's. Extended haplotype analysis of the Greek kindreds and the Italian PD family suggests that the mutations arose independently on different ancestral chromosomes. The finding of the Ala53Thr substitution in four independent PD families and its absence from 314 control chromosomes provides the strongest genetic evidence that this mutation in the human alpha synuclein gene is causative for the PD phenotype in these families.

We have also demonstrated by RT PCR that the mutant allele is transcribed in the lymphoblast cell line of an affected individual from the Italian kindred (Figure 3) (7). Thus, it is reasonable to assume that the mutant protein is indeed expressed.

Example 3.

Since homologous genes that are related to the alpha synuclein protein have been identified in other species, it seemed reasonable to assume that homologues of alpha synuclein would exist in humans as well. In fact, human beta synuclein has previously been described (46), and is approximately 60% similar to alpha synuclein at the protein level.

We set out to identify other related homologues by searching various databases for homologous genes and proteins. Protein sequence databases searched included the NR (non-redundant) and "month" databases of Genbank and Swiss Prot. Nucleotide databases included NR, month, dbstf, GSS (Genome Sequence Service) and EPD (eurkaryotic Promoter Database). Several human clones were identified and characterized as alpha, beta and gamma clones as shown in Figure 7. Potential gamma clones were identified on the basis of homology to known rat and mouse sequences. Although gamma synuclein has been identified in species other than

human, this is the first identification of the corresponding gamma synuclein from humans.

Using two primers sets designed from known database sequences (5'ATGTCTTCAAGAAGGGCTTC3'; 5'CCTTGGTCTTCTCAGCTGCT3' and 5'AGCGTGGATGACCTGAAGAG3'; 5'AGCACAGGTGGACAGGCCAAG3'), we have isolated two BAC clones, 139A20 and 174P13, from a Genome System commercial Bacterial Artificial Chromosome library (St. Louis, MO) which contain the human beta and gamma synuclein genes, respectively. The beta gene contained one clone 139A20 has been sequenced as shown in Figure 8 (SEQ ID NO 11), which contains all coding exon sequences and some additional non-coding intronic sequence. The gamma clone 174P13 has been sequenced and is available in GenBank: accession number AF044311. Sequence from the 5' end is given in Figure 9 (SEQ ID NO 12), and sequence from the 3' end is given in Figure 10 (SEQ ID NO 13). The human alpha synuclein gene has also been sequenced as shown in Figure 11, which provides the sequence of each separate exon region with some additional flanking intronic sequence for each exon. (SEQ ID NOs 14-19)

The three human homologues are highly conserved at the protein level. The alpha and beta human homologues have about 60.4% similarity. And the gamma homologue is about 38.3% and 32.8% similar to the alpha and beta homologues, respectively, based on the portion of the coding sequence

that we have obtained thus far. Thus, it is reasonable to presume that mutations in either the beta or gamma synuclein gene may also result in Parkinson's disease.

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5,047,519	Hobbs, Jr. et al.	September 10, 1991

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47. Polymeropoulos et al. (1997) Science 276:2045-2047, which is relied upon and hereby expressly incorporated by reference herein.

48. Lavedan et al. (1998) in press, which is relied upon and hereby expressly incorporated by reference herein.

49. This application is based on provisional application number 60/505,684 filed June 25, 1997 which is relied upon and hereby expressly incorporated by reference herein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Polymeropoulus, Mihael
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(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 216 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein gene/ exon 4 region

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: 4
(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTAATCAGC AATTTAAGGC TAGCTTGAGA CTTATGTCTT GAATTTGTTT TTGTAGGCTC 60

CAAAACCAAG GAGGGAGTGG TGCATGGTGT GACAACAGGT AAGCTCCATT GTGCTTATAT 120

CAAAGATGAT ATNTAAAGTAT CTAGTGATTA GTGTGGCCCA GTATCAAGAT TCCTATGAA 181

ATTGTAAAACA ATCACTGAGC ATCTAAGAAC ATATC 216

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer #3"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCTAATCAGC AATTTAGGCT AG 22

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer #13"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTATACAAGA ATCTACGAGT C 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(C) INDIVIDUAL ISOLATE: Swiss-Prot P37840

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
35 40 45

Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
50 55 60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
65 70 75 80

Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys

85 90 95

Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
100 105 110

Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
115 120 125

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
130 135 140

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus norvegicus
(C) INDIVIDUAL ISOLATE: Swiss-Prot P37377

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
35 40 45

Val His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
50 55 60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
65 70 75 80

Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Phe Val Lys
85 90 95

Lys Asp Gln Met Gly Lys Gly Glu Glu Gly Tyr Pro Gln Glu Gly Ile
100 105 110

Leu Glu Asp Met Pro Val Asp Pro Ser Ser Glu Ala Tyr Glu Met Pro
115 120 125

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
130 135 140

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 134 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bos taurus
(C) INDIVIDUAL ISOLATE: Swiss-Prot P33567

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val
1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
35 40 45

Val Gln Gly Val Ala Ser Val Ala Glu Lys Thr Lys Glu Gln Ala Ser
50 55 60

His Leu Gly Gly Ala Val Phe Ser Gly Ala Gly Asn Ile Ala Ala Ala
65 70 75 80

Thr Gly Leu Val Lys Lys Glu Glu Phe Pro Thr Asp Leu Lys Pro Glu
85 90 95

Glu Val Ala Gln Glu Ala Ala Glu Glu Pro Leu Ile Glu Pro Leu Met

100 105 110

Glu Pro Glu Gly Glu Ser Tyr Glu Glu Gln Pro Gln Glu Glu Tyr Gln
115 120 125

Glu Tyr Glu Pro Glu Ala
130

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 142 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Serinus canaria
(C) INDIVIDUAL ISOLATE: genbank L33860

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein homologue

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Val Val Ala
1 5 10 15

Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys Thr
20 25 30

Lys Glu Gly Val Leu Tyr Val Gly Ser Arg Thr Lys Glu Gly Val Val
35 40 45

His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Ser Asn
50 55 60

Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys Thr
65 70 75 80

Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Leu Val Lys Lys
85 90 95

Asp Gln Leu Ala Lys Gln Asn Glu Glu Gly Phe Leu Gln Glu Gly Met
100 105 110

Val Asn Asn Thr Gly Ala Ala Val Asp Pro Asp Asn Glu Ala Tyr Glu
115 120 125

Met Pro Pro Glu Glu Glu Tyr Gln Asp Tyr Glu Pro Glu Ala
130 135 140

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Torpedo californica
(C) INDIVIDUAL ISOLATE: Swiss-Prot P37379

(vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein homologue

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asp Val Leu Lys Lys Gly Phe Ser Phe Ala Lys Glu Gly Val Val
1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Gln Asp Ala Ala Glu Lys
20 25 30

Thr Lys Gln Gly Val Gln Asp Ala Ala Glu Lys Thr Lys Glu Gly Val
35 40 45

Met Tyr Val Gly Thr Lys Thr Lys Glu Gly Val Val Gln Ser Val Asn
50 55 60

Thr Val Thr Glu Lys Thr Lys Glu Gln Ala Asn Val Val Gly Gly Ala
65 70 75 80

Val Val Ala Gly Val Asn Thr Val Ala Ser Lys Thr Val Glu Gly Val
85 90 95

Glu Asn Val Ala Ala Ala Ser Gly Val Val Lys Leu Asp Glu His Gly
100 105 110

Arg Glu Ile Pro Ala Glu Gln Val Ala Glu Gly Lys Gln Thr Thr Gln

115 120 125

Glu Pro Leu Val Glu Ala Thr Glu Ala Thr Glu Glu Thr Gly Lys
130 135 140

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer #1F"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACGACAGTGT GTGTAAAGG 19

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer #13R"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AACATCTGTC AGCAGATCTC 20

(2) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 2809 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 139A20 HUMAN BETA SYNUCLEIN GENE

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCGCCGCAGC CGCCGCTCCA TCCCCAGCCC CGGCCCCGCA TCCGGTTTGG AAGGGGGCTG

CAAGTTTGCA AGGGGCCCGG GANAAAAANC GAGCAGTGGC GCTTCCCGCG TCCCCAGGGT

TTCAAGGGAC GCTAGGANTN TCCGCGGCCC TGGAGGTTCG CACTGGGGAG TGGGGTGAGA

TGGGGGGAAA GCGGGAGGGG GCTCAGGGTC CAGAAGGGCN CCGCGGTCTC GGGAGTAGGG

GGGCATNTGC GTCCCGCGGG AGGGGCTGGG GTGAGAGTGC GGGGCCAGTG CACCGGTGCC

CGTGTATCGC CCTCCCCAGG CCGCCAGGAT GGACGTGTTC ATGAAGGGCC TGTCCATGGC

CAAGGAGGGC GTTGTGGCAG CCGCGGAGAA AACCAAGCAG GGGGTCACCG AGGCGGCGGA

GAAGACCAAG GAGGGCGTCC TCTACGTCGG TGGGCNGGGG GCNGGGTTTC TGGGGCTGCA

GGGCTGGGGG TCCCCCTACA GTGTGGAGCT GGGGCCGGGT CCCGGGGAGG GGGGTTCTGG

GCAAGATAAT ATNANTCAGC AGATGGGGCN AGGTCANCAN GGGTCATAAG GGACATACCC

ANCCCATAGA ANCCTGGGTC TGTATCCGGA AATGGGGACA CGGGGCGGGC TGATGAGGTG

GGGGGCTCCA NCTGAAAGGC CAGGGACCAN TGCANTNATA AAANCACACA NCCTCCTTTT

TCTTATCTTT TTTACCATTA TTAATAGTTA TCTGGTGTTG AACACTTTCT GTATGCCAAG

TACTGGGTAA AATGTCATAA CATCCATTTC CTCATGTAAT GCTTCCGCCC ATTCTACAGG

TAAGGGAAAC TGGGCTTCCC ATTGGTAGNT AAATTTTAGG TTCAGAAAGG CTTGAATTGA

ATGTCAGTTC AGCCAATTTC TTAGTGGTGG AACCAAACTG AGTTCCATCC GTGAAACGGG

GACAATAACA GCACCCGCTT CCCAGGGCTG GGGAAAAGTG AAGTGCAGCG GGGCAGGCAG

AGGACTTGAC ACAGCACTGG CCCTCAGCCA ACATCCACTA GAGGGGTGGG GTATCGCATC

AGGTGGGAGA GAACTGCAAC CCTTGCAGAC AGAGGTGTGG GGCCCAGTGC AGTGATAAGA

CGGGGGTTAA CATGGGGGTG CAGGTTGTAG GATNTGGGGA CCCAAGGAGG CAGTGACGGG

GCCAGGATGC CCACTCTGTA ATCACCATGC TGTGCTGGAG TTTCTGTTCC CTCAGCGCAG

AGTCCTTAAA TGTGCCGCTT TTTCTNCCCT GCAGGAAGCA AGACCCGAGA AGGTGTGGTA

CAAGGTGTGG CTTCAGGTAC TAGCCCAGCC CTGGCACCAG CCCTTCTCTC AMTTAGGCGG

ATGATCTGGC CGGGAACCAG AGGGCGGGGG CGGGGGAGAC TCCCAAGGCT TCTGCGGGAA

TGCTCCGTGG GGAGGGCAGG CCCTGGGATA CTACAAGGCA GGGCATCGGT GTTTCCCCCT

GGCTCCCAAA CCCCTTCCTC AACCCCCTCC CTGCTCCAGT GGCTGAAAAA ACCAAGGAAC

AGGCCTCACA TCTGGGAGGA GCTGTGTTCT CTGGGGCAGG GAACATCGCA GCAGCCACAG

GACTGGTGAA GAGGGAGGAA TTCCCTACTG ATCTGAAGGT AAGCGATCCT TCTGACCCGC

ACATGCAGGC AAACACACAC ACACACACAC ACACACACCN GGCACACAAA TAAACCTGTC

ACCATCCCCG CCCCCCTAAT CCTGCCACCA GCTTGGAACA CAAGCCACTT TGCCTCCCAT

CCTGCNGGCC CGTGCTAGAC TCAGCTCAGA ATGCATCTGA ATAANGGCGT GCATGGGTGT

GACGCTCCCG GTGATGGGGA CCCAGACCTG GCTGTCTGCG TGTATCCTGC TTGCCAGCGT

GACCCATATG ACTTCTGGCC ACGTCTGCAT GTGTCAATGA TTGTTCATTC ATTTCTTTTC

ATTCAACAAA TATCCATGCC ANANCCAGCC CTGTCCTTGA GCTTCCAGNT CCCTTTCAGC

CNAGGGGAGC NTGAGGGTTA TTTTTGGGGT CCCGATGCCC AGCACAGAGC CTGACACAAA

GGATGAGGCA TAAGCTGGTG ANTGAGTATC CAAATGGTGG AAGTGTGGAG GNTGCCAGGC

ATTGGGGGAG CGGCGTGGAG AGCCAGCTCC CCAATCCATG CTGCCACTTC AACTGTGATT

CGGGGGAATT TCCCCCTTCA CCTCCATCCC ACTTCCAAGG CACTCCAAAT AAATAACTGA

ATTAGAAATT ATCCTTGTTT TGCCAACCCA CCCTAGCCTT CCCCACTCCA ACCCACCCAA

AGCTTACCAC TGTGGGAATT TGGGGGGCAT CCTGGCTGTC CTCACGAGTC CTGACCTTTT

CTGCCCACAG CCAGAGGAAG TGGCCCAGGA AGCTGCTGAA GAACCACTGA TTGAGCCCCT

GATGGAGCCA GAAGGGGAGA GTTATGAGGA CCCACCCCAG GAGGAATATC AGGAGTATGA

GCCAGAGGCG TAGGGGCCCA GGAGAGCCCC CACCAGCAGC ACAATTCTGT CCCTGTCCCT

GCCCCGCCCC CCAGAGCCAG GGCTGTCCTT AGACTCCTTC TCCCCAATCA CGAGATCTTC

CTTCCGCTCT GAGGCAACCC CCTCGGAGCC TGTGTTAGTG TCTGTCCATC TGTCTGTCCT

ACCCGCCCGC GTCCAACCCC GGGGCATGGA CAGGGCCAGG GTTGCGGTCG CGGCTGGGAG

CCTCGCCCCT CCAGTGTTGC CTCCTCCCAT CCAGCGTCTG CGCG

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 223 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 174P13 HUMAN GAMMA SYNULEIN GENE, 5' END

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGGGAGATCC AGCTCCGTCC TGCCTGCAGC AGCACAACCC TGCACACCCA CCATGGATGT

CTTCAAGAAG GGCTTCTCCA TCGCCAAGGA GGGNGTGGTG GGTGCGGTGG AAAAGACCAA

GCAGGGGGTG ACGGAAGCAG CTGAGAAGAC CAAGGAGGGG GTCATGTATG TGGGATTACA

TTTTTTTTTT AAAGAAAGAA TAAATTAATT GTGATTAAAG TTG

(2) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 677

(B) TYPENUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 174P13 HUMAN GAMMA SYNULEIN GENE, 3' END

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTTTTTNAGG GGGGAAAACA GGGAATANAA AAANANGGGG GGGGGTTTTT NNGGGGGGGG

GGGGAAAANG GTTNGGGGGN NAACCNAAAN AAANNCCNAN GGGGGGGGNN ANTNAANTTT

TGGGAACCCA AAGCCCNAGG AGGATTTTTN GTNAANAACG TNACCTCNAG TGGGNCGAGG

AAGACCAAGG AAANGCCCAA CNCGGTTGAN CGAGGCTGTG GTGAACANCG TNCAACNCTG

TGCCCNCCAA NANCGTGGAG GNGGCGGAGA ACATCSCGGT CACCTCCGGG GTGGTGCGCM

AGGAGGACTT GAGGCCATCT KCCCCCCMAC AGGAGGGTGT GGCATCCMAA GARAAAGAGG

AAGTGGCAGA GGAGGCCCAG AGTGGGGGAR ACTAGAGGGC TACAGGCCAG CGTGGATGAC

CTGAAGAGCG CTCCTCTGCC TTGGACACCA TCCCCTCCTA GCACAAGGAG TGCCCGCCTT

GAGTGACATG CGGCTGCCCA CGCTCCTGCC CTCGTCTTCC TGGCCACCCT TGGCCTGTCC

ACCTGTGCTG CTGCACCAAC CTCACTGCCC TCCCTCGGCC CCACCCACCC TCTGGTCCTT

CTGACCCCAC TTATGCTGCT GTGAATTTTT TTTTTAAATG ATTCCAAATA AAACTTGAGC

CCACTCCAAA AAAAAAA

(2) INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 1181 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene: exons 1 and 2 plus flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AATTTCAGCG ATGCGAGGGC AAAGCGCTCT CGGCGGTGCG GTGTGAGCCA CCTCCCGGCG

CTGCCTGTCT CCTCCAGCAG CTCCCCAAGG GATAGGCTCT GCCCTTGGTG GTCGACCCTC

AGGCCCTCGN TCTCCCAGGN CGACTCTGAC GAGGGGTAGG GGGTGGTCCC CNGGAGGACC

CAGAGGAAAG GCNGGGACAA GAAGGGAGGG GAAGGGGAAA GAGGAAGAGG CATCATCCCT

AGCCCAACCG CTCCCGATCT CCACAAGAGT GCTCGTGACC CTAAACTTAA CGTGAGGCGC

AAAAGCGCCC CAACCTTTTC CCGCCTTGNN CCAGGCAGGC GGCTGGAGTT GATGGCTCAC

CCCGCGCCCC CTGCCCCATC CCCATCCGAG ATAGGGACGA GGAGCACGCT GCAGGGAAAG

CAGCGAGCGC CGGGAGAGGG GCGGGCAGAA GCGCTGACAA ATCAGCGGTG GGGGCGGAGA

GCCGAGGAGA AGGAGAAGGA GGAGGACTAG GAGGAGGAGG ACGGCGACGA CCAGAAGGGG

CCCAAGAGAG GGGGCGAGCG ACCGAGCGCC GCGACGCGAA GTGAGGTGCG TGCGGGCTCA

GCGCAGACCC CGGCCCGGCC CCTCCTGAGA GCGTCCTGGG CGCTCCCTCA CGCCTTGCCT

TCAAGCCTTC TGCCTTTCCA CCCTCGTGAG CGGAGAACTG GGAGTGGCCA TTCGACGACA

GGTTAGCGGG TTTGCCTCCC ACTCCCCCAG CCTCGCGTCG CCGGCTCACA GCGGCCTCCT

CTGGGGACAG TCCCCCCCGG GTGCCCCTCC GCCCTTCCTG TGCGCTCCTT TTCCTTCTTC

TTTCCTATTA AATATTATTT GGGAATTGTT TAAATTTTTT TTTTAAAAAA AGAGAGAGGC

GNGGAGGAGT CGGAGTTGTG GAGAAGCAGA GGGACTCAGG TAAGTACCTG TGGATCTAAA

CGGGNGTCTT TTGGAAATCC TGGAGAACGC CGGATGGAGA CGAATGGTCG TGGGNACCGG

GAGGGGGTGG TGCTGCCATG AGGACCGCTG GGCCAGGTCT CTGGGAGGTG AGTACTTGTC

CTTTGGGGAG CTAAGGAAAG AGACTTGACC TGGCTTTCGT CCTGCTTCTG ATATTCCCTT

CTCCACAAGG GCTGAGAGNT TAGGCTGCTT CTCCGGGATC C

(2) INFORMATION FOR SEQ ID NO:15

(i)SEQUENCE CHARACTERISITCS

(A)LENGTH: 536 base pairs

(B)TYPE: NUCLEIC ACID

(C)STRANDEDNESS:DOUBLE

(D)TOPOLOGY: LINEAR

(ii)MOLECULAR TYPE: DNA (genomic)

(iii)HYPOTHETICAL: NO

(iv)ANTI-SENSE: NO

(vii)IMMEDIATE SOURCE:

(A)CLONE: human alpha synuclein gene/ exon 3 plus flanking intron sequences

(viii)POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO 15:

CTTAAAAGAG TCTCACACTT TGGAGGGTTT CTCATGATTT TTCAGTGTTT TTTGTTTATT

TTTCCCCGAA AGTTCTCATT CAAAGTGTAT TTTATGTTTT CCAGTGTGGT GTAAAGAAAT

TCATTAGCCA TGGATGTATT CATGAAAGGA CTTTCAAAGG CCAAGGAGGG AGTTGTGGCT

GCTGCTGAGA AAACCAAACA GGGTGTGGCA GAAGCAGCAG GAAAGACAAA AGAGGGTGTT

CTCTATGTAG GTAGGTAAAC CCCAAATGTC AGTTTGGTGC TTGTTCATGA GTGATGGGTT

AGGATAACAA TACTCTAAAT GCTGGTAGTT CTCTCTCTTG ATTCATTTTT GCATCATTGC

TTGTCAAAAA GGTGGACTGA GTCAGAGGTA TGTGTAGGTA GGTGAATGTG AACGTGTGTA

TNTGAGCTAA TAGTAAAAAT GCGACTGTTT GCTTTTCAGA TTTTTAATTT TGCCTAATAT

NTATGACTTN TTAAAATGAA TGTTTCTGTA CTACATAATT CTATNTCAGA GACAGT

(2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 650 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exon 4 plus flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGCAGGTCA ACGGATCTGT CTCTAGTGCT GTACTTTTAA AGCTTCTACA GTTCTGAATT

CAAAATTATC TTCTCACTGG GCCCCGGTGT TATCTCATTC TTTTTTCTCC TCTGTAAGTT

GACATGTGAT GTGGGAACAA AGGGGATAAA GTCATTATTT TGTGCTAAAA TCGTAATTGG

AGAGGACCTC CTGTTAGCTG GGCTTTCTTC TATNTATTGT GGTGGTTAGG AGTTCCTTCT

TCTAGTTTTA GGATATATAT ATATATTTTT TCTTTCCCTG AAGATATAAT AATATATATA

CTTCTGAAGA TTGAGATTTT TAAATTAGTT GTATTGAAAA CTAGCTAATC AGCAATTTAA

GGCTAGCTTG AGACTTATGT CTTGAATTTG TTTTTGTAGG CTCCAAAACC AAGGAGGGAG

TGGTGCATGG TGTGGCAACA GGTAAGCTCC ATTGTGCTTA TATCAAAGAT GATATNTAAA

GTATCTAGTG ATTAGTGTGG CCCAGTATCA AGATTCCTAT TGAAATTGTA AAACAATCAC

TGAGCATCTA AGAACATATC AGTCTTATTG AAACTGAATT CTTTATAAAG TATTTTTAAA

TAGGTAAATA TTGATTATAA ATAAAAAATA TACTTGCCAA GAATAATGAG

(2) INFORMATION FOR SEQ ID NO:17

(i)SEQUENCE CHARACTERISITCS

(A)LENGTH: 504 base pairs

(B)TYPE: NUCLEIC ACID

(C)STRANDEDNESS:DOUBLE

(D)TOPOLOGY: LINEAR

(ii)MOLECULAR TYPE: DNA (genomic)

(iii)HYPOTHETICAL: NO

(iv)ANTI-SENSE: NO

(vii)IMMEDIATE SOURCE:

(A)CLONE: human alpha synuclein gene/ exon 5 plus flanking intron sequences

(viii)POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATATCTTAGC CAAGATTCAA TGTTTGGTTG AACCACACTC ACTTGACATC TTGGTGGCTT

TTGTTTCTTC TGACCACTCA GTTATCTATG GCATGTGTAG ATACAGGTGT ATGGAANCGA

TGGCTAGTGG AAGTGGAATG ATTTTAAGTC ACTGTTATTC TACCACCCTT TAATCTGTTG

TTGCTCTTTA TTTGTACCAG TGGCTGAGAA GACCAAAGAG CAAGTGACAA ATGTTGGAGG

AGCAGTGGTG ACGGGTGTGA CAGCAGTAGC CCAGAAGACA GTGGAGGGAG CAGGGAGCAT

TGCAGCAGCC ACTGGCTTTG TCAAAAAGGA CCAGTTGGGC AAGGTATGGC TGTGTACGTT

TTGTGTTACA TTTATAAGCT GGTGAGATTA CGGTTCATTT TCATGTGAAG CCTGGAGGCA

GGAGCAAGAT ACTTACTGTG GGGAACGGCT ACCTGACCCT CCCCTTGTGA AAAAGTGCTA

CCTTTATATT GGTCTTGCTT GTTT

(2) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 727 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene exons 1 and 2 plus flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AAAAGTTTAC ATACTTTGAG GTTGATAACC CATGTTGCCG CAATGTTTCC CCGGAGGCAT

TGTGGAGTTT AGAATGCCAG TAGTAATATT AAGGTGTGCC ATTTTCAAGA TCCGTGGCCA

ACATCCCTAT ATGTAAGATT TTTCCAAAAC ATGGTTCTGA TTTTTAAAAG TGAAAAATGC

TACTTCATCA TGTTCTTTTT GTGCTTCTTA CTTTAAATAT TAGAATGAAG AAGGAGCCCC

ACAGGAAGGA ATTCTGGAAG ATATGCCTGT GGATCCTGAC AATGAGGCTT ATGAAATGCC

TTCTGAGGTA GGAGTCCAAG CTGAATCTTT CTAACAAGAC AGTACCAAAA ACCTGTCATT

GTCACATTTC TCTTTCATTA GTGCTTAGTG AGAATCATTT GCTCTCTACA TGCTCATTA

GTGGACAACT TGCAAGTTAA GAATAGTTTT TACATTTTTA AAGGGTCCTT AAAAAAAAAG

AGGAGGAGGA AGATGAAGAA GAGGAAGAAA GGATGTAAAA GAAATCATAT GTAGTCCACA

TAGCTTAATA TACNTACTAC TTGACCCTTT ACAGGAAAAG CTTTACTAAC CCCTGCATTA

GAGAATATAT TTTTTTGCAA AAACATTGAT TGTAAATTTT AGTGTAAAGT GGGGAGCCAT

TTCCTATCTC ATTGGCTGTC CAGTGCTGAT GCGTAATTGA AACTTATACT AACAGTGTGT

GCTGTCT

(2) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 1596 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exon 7 plus flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTTTGATTTT TCTAATATTA GGAAGGGTAT CAAGACTACG AACCTGAAGC CTAAGAAATA

TCTTTGCTCC CAGTTTCTTG AGATCTGCTG ACAGATGTTC CATCCTGTAC AAGTGCTCAG

TTCCAATGTG CCCAGTCATG ACATTTCTCA AAGTTTTTAC AGTGTATCTC GAAGTCTTCC

ATCAGCAGTG ATTGAAGCAT CTGTACCTGC CCCCACTCAG CATTTCGGTG CTTCCCTTTC

ACTGAAGTGA ATACATGGTA GCAGGGTCTT TGTGTGCTGT GGATTTTGTG GCTTCAATCT

ACGATGTTAA AACAAATTAA AAACACCTAA GTGACTACCA CTTATTTCTA AATCCTCACT

ATTTTTTTGT TGCTGTTGTT CAGAAGTTGT TAGTGATTTG CTATCATATA TTATNAGATT

TTTAGGTGTC TTTTAATGAT ACTGTCTAAG AATAATGACG TATTGTGAAA TTTGTTAATA

TATATNATAC TTAAAAATAT GTGAGCATGA AACTATGCAC CTATAATACT AAATATGAAA

TTTTACCATT TTGCGATGTG TTTTATTCAC TTGTGTTTGT ATATNAATGG TGAGAATTAA

AATAAAACGT TATCTCATTG CAAAAATATT TTATTTTTAT CCCATCTCAC TTTAATAATA

AAAATCATGC TTATAAGCAA CATGAATTAA GAACTGACAC AAAGGACAAA AATATAAAGT

TATTAATAGC CATTTGAAGA AGGAGGAATT TTAGAAGAGG TAGAGAAAAT GGAACATTAA

CCCTACACTC GGAATTCCCT GAAGCAACAC TGCCAGAAGT GTGTTTTGGT ATGCACTGGT

TCCTTAAGTG GCTGTGATTA ATTATTGAAA GTGGGGTGTT GAAGACCCCA ACTACTATTG

TAGAGTGGTC TATTTCTCCC TTCAATCCTG TCAATGTTTG CTTTACGTAT TTTGGGGAAC

TGTTGTTTGA TGTGTATGTG TTTATAATTG TTATACATTT TTAATTGAGC CTTTTATTAA

CATATATTGT TATTTTTGTC TCGAAATAAT TTTTTAGTTA AAATCTATTT TGTCTGATAT

TGGTGTGAAT GCTGTACCTT TCTGACAATA AATAATATNC GACCATGAAT AAAAAAAAAA

AAAAAGTGGG TTCCCGGGAA CTAAGCAGTG TAGAAGATGA TTTTGACTAC ACCCTCCTTA

GAGAGCCATA AGACACATTA GCACATATTA GCACATTCAA GGCTCTGAGA GAATGTGGTT

AACTTTGTTT AACTCAGCAT TCCTCACTTT TTTTTTTTAA TCATCAGAAA TTCTCTCTCT

CTCTCTCTTT TTCTCTCGCT CTCTTTTTTT TTTTTTTTTT TTTTACAGGA AATGCCTTTA

AACATCGTTG GGAACTACCA GAGTCACCTT AAAGGGAGNA TCAATTCTCT AGGACTGGAT

AAAAATTTCA TGGGCCTCCT TTAAAATGTT GCCCAAATAT ATGGAATTCT AGGGGTTTTT

CCNTAGGGGG AAGGGTTTTT TCTCTTTTCN GGGGAGGATC CTTTTAACNC CCCNGGGGGG

NGCCCGGAAA ATAAACTTGG NGGGGGGGNA AAACTT

WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising a nucleotide sequence encoding a mutated human synuclein protein or homologue thereof.

2. The isolated nucleic acid of claim 1 wherein said mutated synuclein protein is selected from the group consisting of alpha, beta and gamma synuclein proteins.

3. The isolated nucleic acid of claim 2 wherein said mutated synuclein protein is the alpha synuclein protein.

4. The isolated nucleic acid of claim 3 wherein said nucleotide sequence contains at least one mutation at base pair position 209.

5. The isolated nucleic acid of claim 4 wherein said mutation at position 209 is a change from guanine to adenine.

6. The isolated nucleic acid of claim 5 having the sequence given in SEQ ID NO. 1.

7. An oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.

8. The oligonucleotide of claim 7 wherein said mutation is at base pair position 209 in the synnuclein gene.

9. The oligonucleotide of claim 8 wherein said mutation is a change from guanine to adenine.

10. A vector comprising the isolated nucleic acid of claim 1.

11. A host cell comprising the vector of claim 10.

12. A method of affecting characteristics of Parkinson's Disease, comprising of expressing nucleic acids which are implicated in disease development in cultured cells through the use of expression vectors.

13. The method of claim 12 wherein the said nucleic acid is selected from the group consisting of alpha, beta, and gamma synuclein genes.

14. The method of claim 13 wherein the said nucleic acid encodes the mutated alpha synuclein protein.

15. The method in claim 14 wherein the said mutated alpha synuclein protein contains at least one mutation at base pair 209.

16. The method of claim 15 wherein said mutation at position 209 is a change from guanine to adenine.

17. An isolated human synuclein protein or peptide containing at least one mutation.

18. The isolated human synuclein protein or peptide of claim 17 wherein said protein or peptide is selected from the group consisting of

the human alpha, beta and gamma synuclein proteins or fragments thereof.

19. The isolated human synuclein protein or peptide of claim 18 having the sequence given in SEQ ID NO 5.

20. The isolated human synuclein protein or peptide of claim 19 wherein said protein or peptide is the alpha synuclein gene or a fragment thereof.

21. The isolated protein or peptide of claim 20, wherein said mutation is at amino acid position 53.

22. The isolated protein or peptide of claim 21, wherein said mutation is an alanine to threonine substitution.

23. An antibody specific for the protein or peptide of claim 17.

24. A method of detecting subjects at increased risk for Parkinson's Disease, comprising:

obtaining a sample comprising nucleic acids, proteins or tissues from the subjects, and

detecting in the nucleic acids, proteins or tissues the presence of a mutation which is associated with Parkinson's disease,

thereby identifying subjects at increased risk for the disease.

25. The method of claim 24 wherein said mutation is located on human chromosome four.

26. The method of claim 25 wherein said mutation is located in the alpha synuclein gene.

27. The method of claim 26 wherein said mutation causes an amino acid substitution at position 53.

28. The method of claim 27 wherein said mutation causes an alanine to threonine substitution at position 53.

29. The method of claim 24 wherein said detecting step comprises combining a nucleotide probe which selectively hybridizes to a nucleic acid containing said mutation, and detecting the presence of hybridization.

30. The method of claim 29 wherein said nucleotide probe is an oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.

31. The method of claim 30 wherin the mutation of said oligonucleotide is at base pair position 209 in the alpha synuclein gene.

32. The method of claim 31 wherein the mutation of said oligonucleotide is a change from guanine to adenine.

33. The method of claim 24 wherein said detecting step comprises amplifying a nucleic acid product comprising said mutation, and detecting

the presence of said mutation in the amplified product.

34. The method of claim 33 wherein said detecting step comprises selectively amplifying a nucleic acid product comprising said mutation, and detecting the presence of amplification.

35. The method of claim 34 wherein said amplifying step comprises at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids.

36. The method of claim 35 wherein said amplifying step uses two oligonucleotides.

37. The method of claim 36 wherein said two oligonucleotides have the sequences of SEQ ID NOs 2 and 3.

38. The method of claim 24 wherein said detecting step comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of said sample of nucleic acids.

39. The method of claim 38 wherein said restriction endonuclease site is recognized by *Tsp*451.

40. The method of claim 24 wherein said detecting step comprises chain termination with a labeled dideoxynucleotide.

41. An oligonucleotide complementary to a nucleic acid sequence

which flanks a mutation in the synuclein gene that is associated with predisposition to Parkinson's disease, wherein said oligonucleotide may be used in diagnostic screens in the amplification of a nucleic acid sequence comprising said mutation.

42. The oligonucleotide of claim 41 having the sequence of SEQ ID NO 2.

43. The oligonucleotide of claim 41 having the sequence of SEQ ID NO 3.

44. The method of claim 24 wherein said detection step comprises identification of said mutations with an antibody.

45. The method of claim 44 wherein said antibody is directed against an isolated human synuclein protein or peptide containing at least one mutation.

46. The method of claim 45 wherein said isolated human synuclein protein or peptide is selected from a group consisting of the human alpha, beta, and gamma synuclein proteins or fragments thereof.

47. The method of claim 46 wherein said isolated human synuclein protein or peptide has the mutated sequence given in SEQ ID NO 5.

48. The method of claim 47 wherein said mutation is at amino acid position 53.

49. The method of claim 48 wherein said mutation is an alanine to threonine substition

50. A diagnostic kit comprising the oligonucleotide of claim 41.

51. A diagnostic kit comprising the oligonucleotide of claim 42.

52. A diagnostic kit comprising the oligonucleotide of claim 43.

53. A diagnostic kit comprising the oligonucleotide of claim 7.

54. A diagnostic kit comprising the oligonucleotide of claim 8.

55. A diagnostic kit comprising the oligonucleotide of claim 9.

56. A diagnostic kit comprising the antibody of claim 23.

57. An isolated nucleic acid comprising a mutation in a human synuclein gene or homologue thereof.

58. The isolated nucleic acid of claim 57 wherein said synuclein gene is the alpha synuclein gene.

59. The isolated nucleic acid of claim 58 wherein said mutation occurs at base pair position 209.

60. The isolated nucleic acid of claim 59 wherein said mutation is

a change from guanine to adenine.

61. The isolated nucleic acid of claim 60 having the sequence given in SEQ ID NO 1.

62. A transgenic animal which expresses a mutated synuclein protein, wherein said animal may be used as an animal model for Parkinson's disease.

63. The transgenic animal of claim 62, wherein said mutated synuclein protein is an alpha synuclein protein.

64. A method of screening a compound for the ability to reverse the self-aggregation of synuclein proteins, comprising exposing an aggregate of synuclein proteins to a test compound and observing whether or not the aggregate is dissolved.

65. The method of claim 64 wherein said test compound is a synuclein peptide.

66. The method of claim 65 wherein said peptide comprises a mutation.

67. The method of claim 64 wherein said test compound is an antibody.

68. The method of claim 64, wherein said observing step comprises

Congo red staining, electron microscopy or CD spectrometry.

69. The method of claim 64 wherein said protein aggregate is located within an animal.

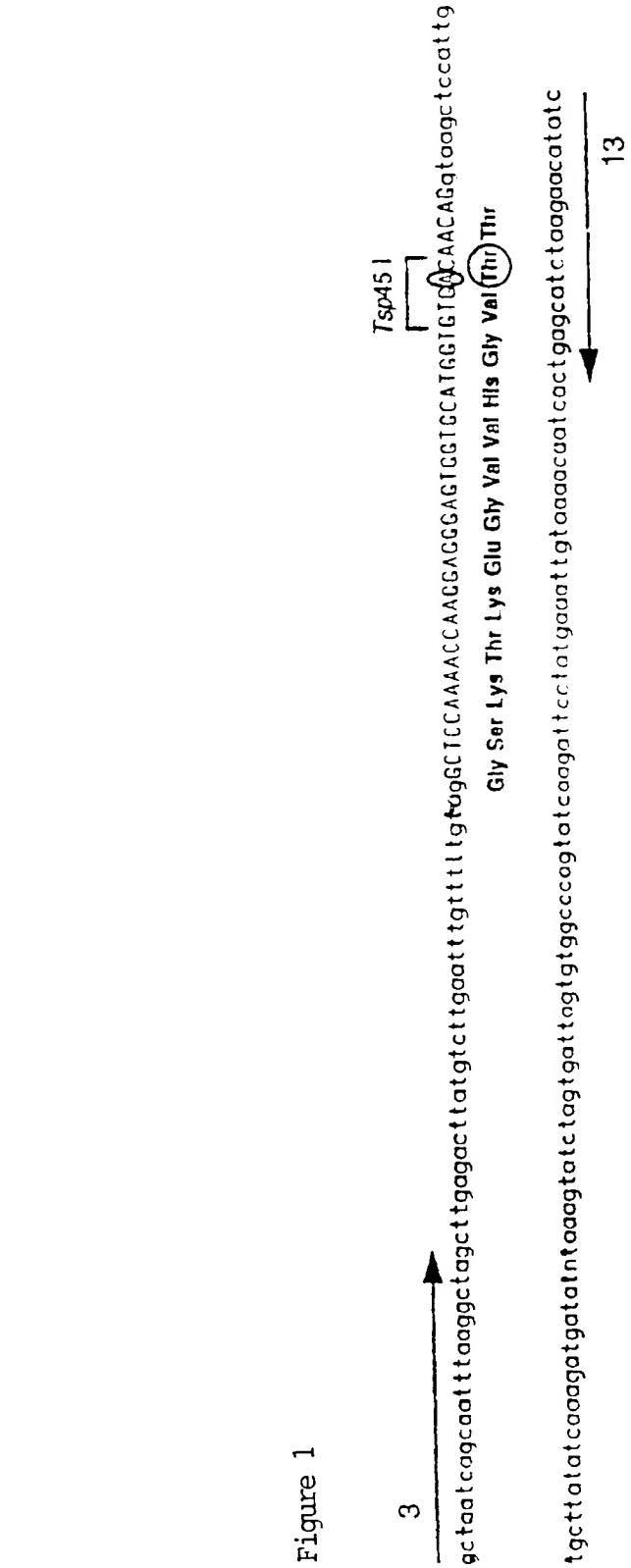
70. A method of screening a compound for the ability to inhibit the self-aggregation of synuclein proteins, comprising exposing a population of synuclein proteins to a test compound under conditions which promote self-aggregation in the absence of said compound and observing whether or not self-aggregation of said proteins is inhibited.

71. The method of claim 70 wherein said test compound is a synuclein peptide.

72. The method of claim 71 wherein said peptide comprises a mutation.

73. The method of claim 70 wherein said test compound is an antibody.

74. The invention substantially as disclosed and described.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3
13
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Gly Ser Lys Thr Lys Glu Gly Val Val His Gly Val Thr Thr
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Figure 1

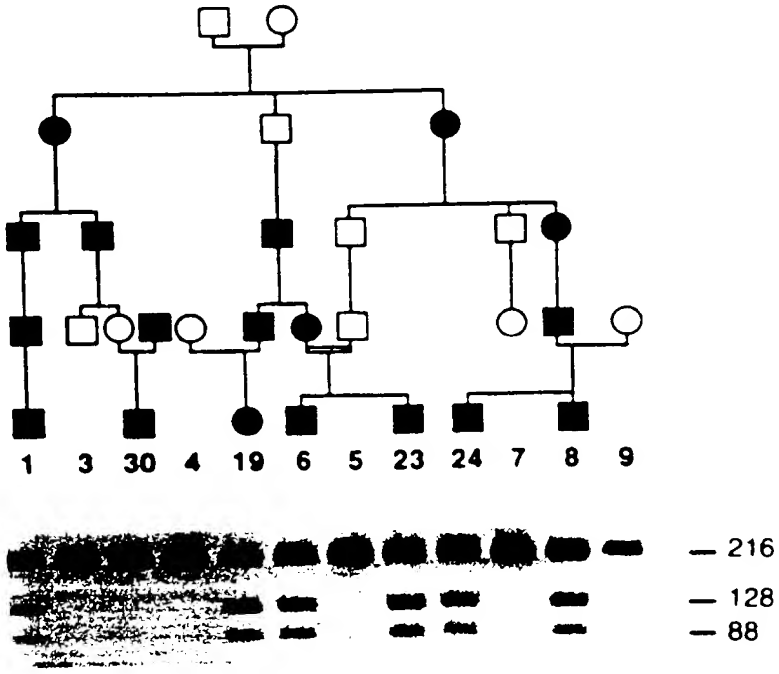

FIG. 2

Figure 4

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1   MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKT----------KEGVLY  Homo sapiens
1   MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKT----------KEGVLY  Rattus norvegicus
1   MDVFMKGLSMAKEGVVAAAEKTKQGVTEAAEKT----------KEGVLY  Bos taurus
1   MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKT----------KEGVLY  Serinus canaria
1   MDVLKKGFSFAKEGVVAAAEKTKQGVQDAAEKTKQGVQDAAEKTKEGVMY  Torpedo californica

    40                  50                  60                  70                  80
40  VGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIA  Homo sapiens
40  VGSKTKEGVVHGVTTVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGNIA  Rattus norvegicus
40  VGSKTKEGVVQGVASVAEKTKEQASHLGGAVPSGAG----------NIA  Bos taurus
40  VGSRTKEGVVHGVTTVAEKTKEQVSNVGGAVVTGVTAVAQKTVEGAGNIA  Serinus canana
51  VGTKTKEGVVQSVNTVTEKTKEQANVVGGAVVAGVNTVASKTVEGVENVA  Torpedo californica

    90                  100                 110                 120
90  AATGFVKKDQLGK--NEEGAPQ--EGI---LED--MPVDPDNEAYEMPS  Homo sapiens
90  AATGFVKKDQMGK--GEEGYPQ--EGI---LED--MPVDPSSEAYEMPS  Rattus norvegicus
79  AATGLVKKEEFPT-DLKPEEVAQ--EAAEEPLIE--PLMEPEGESYEEQP  Bos taurus
90  AATGLVKKDQLAKQN--EEGFLQ--EGM----VNNTGAAVDPDNEAYEMPP  Serinus canaria
101 AASGVVKLDEHGR-EIPAEQVAEGKQTTQEPLVE--ATEATE--------  Torpedo californica

    130                 140
130 EEGYQDYEPEA  Homo sapiens
130 EEGYQDYEPEA  Rattus norvegicus
124 QEEYQEYEPEA  Bos taurus
133 EEEYQDYEPEA  Serinus canaria
140 -------ETGK  Torpedo californica
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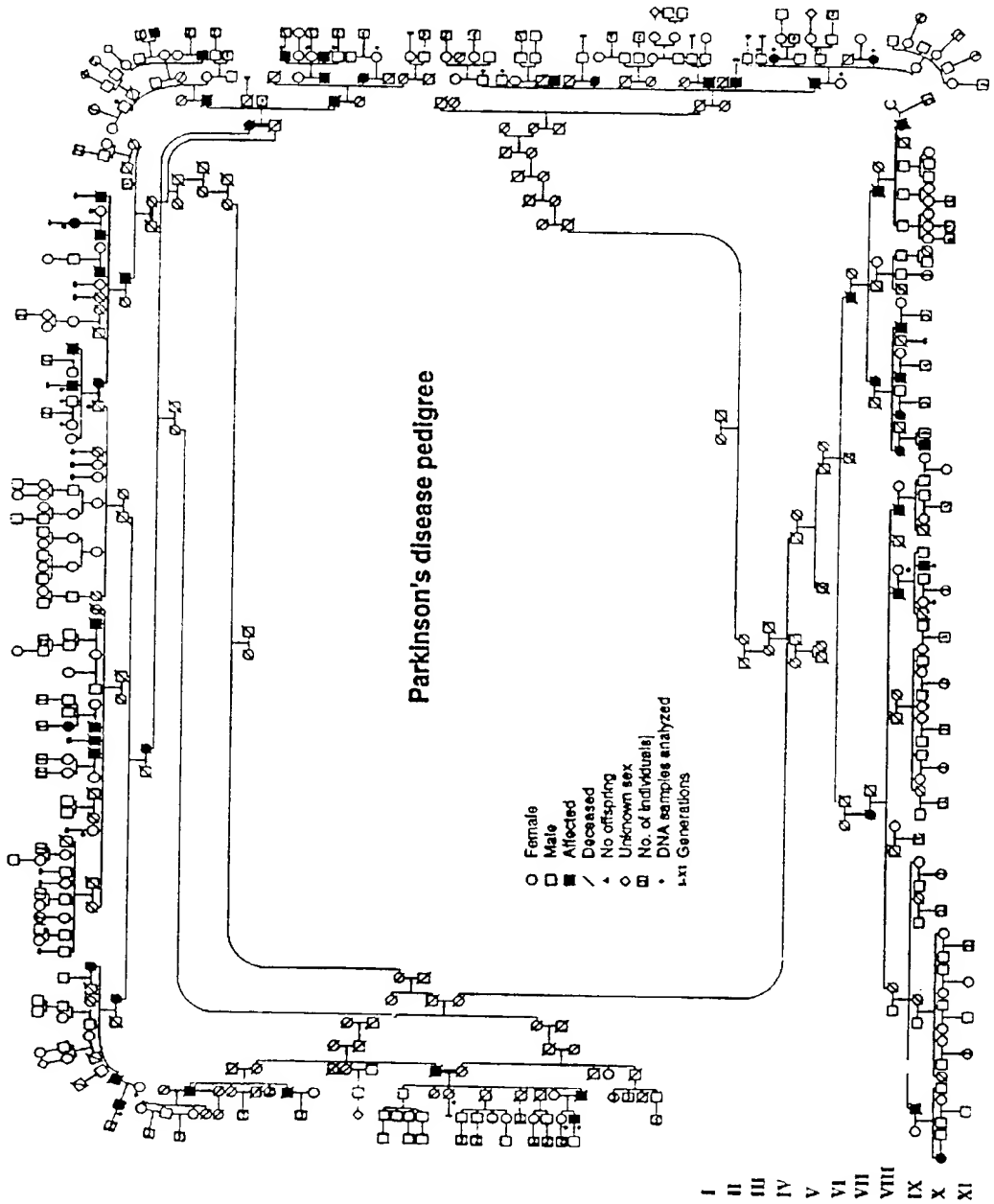

Parkinson's disease pedigree
Female
Male
Affected
Deceased
No offspring
Unknown sex
No. of individuals
DNA samples analyzed
Generations
I
II
III
IV
V
VI
VII
VIII
IX
X
XI

Figure 5

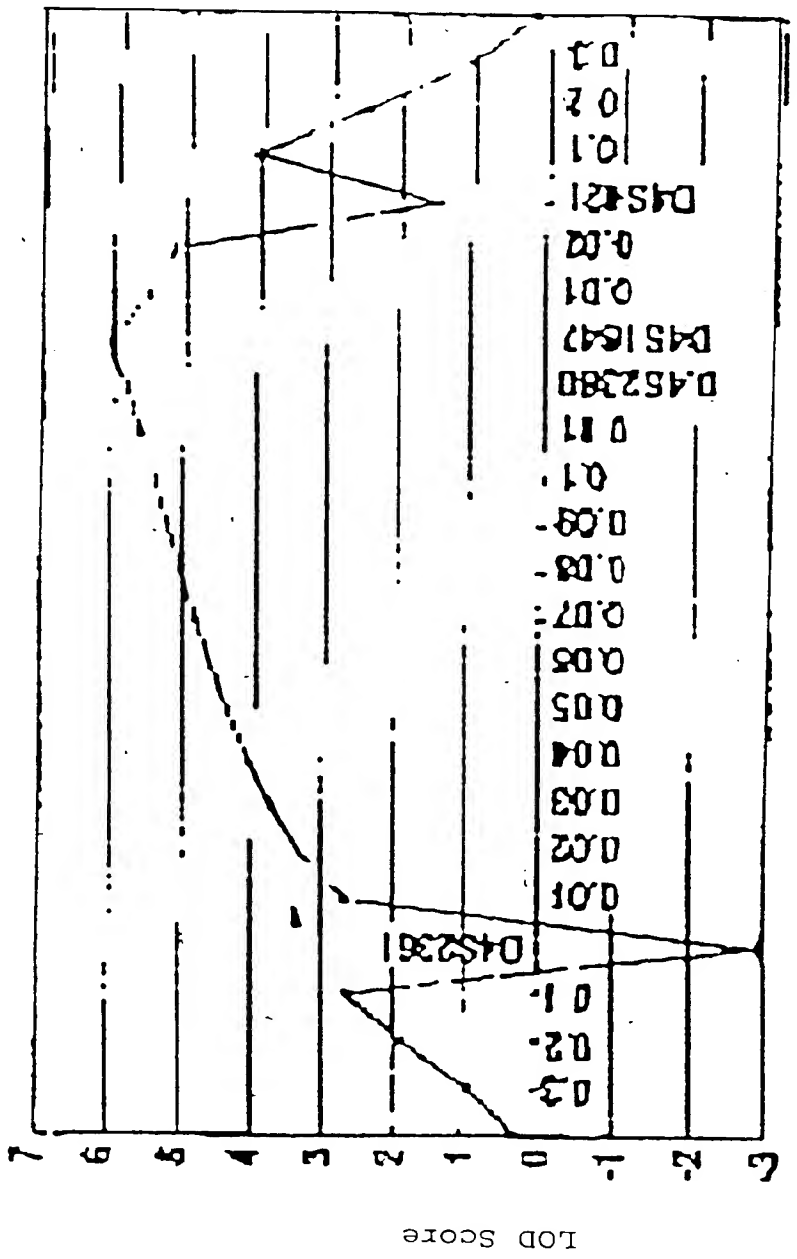
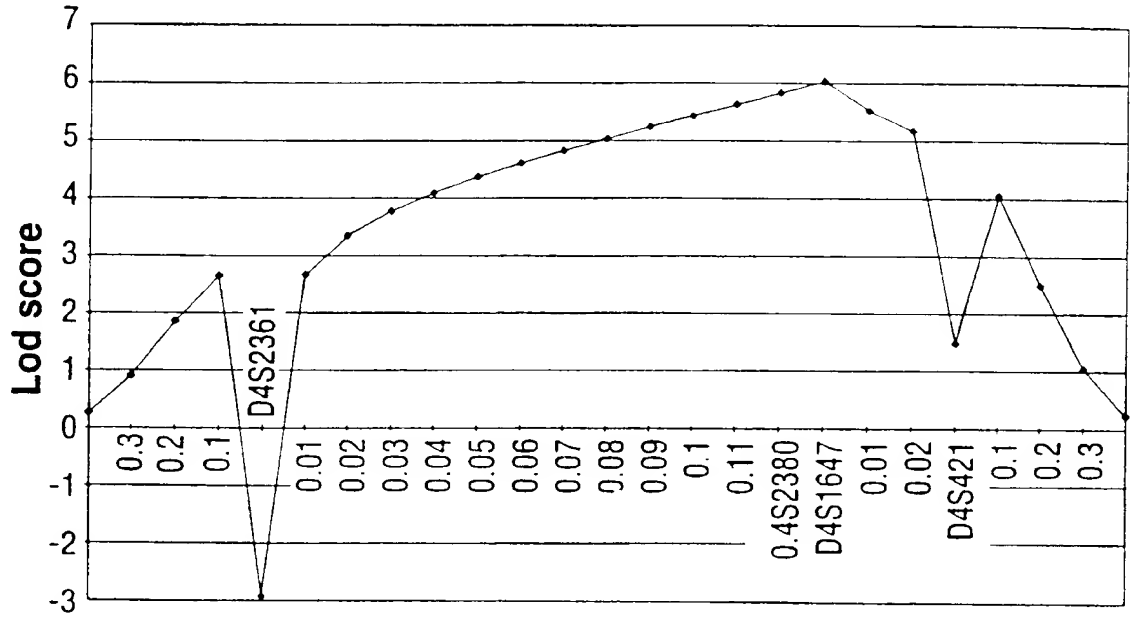


Figure 6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0.09
0.1
0.11
0.4S2380
D4S1647
0.01
0.02
D4S421
0.1
0.2
0.3

FIG. 6

Figure 7/1

clone	5'	3'	gene
109979	T84229	T88834	alpha
111088	T83410		alpha
111090	T83411	T81593	alpha
130048	R11619	(R19409)	alpha
135534	R31354	R32856	alpha
141246	R66663	R67383	alpha
145594	R76091	R77746	alpha
171905	H19290	H19291	beta
172284	H19556	H19474	beta
172749		H19685	beta
175546		H41126	beta
193174	H47503	H47504	alpha
210768	H66914	H66869	alpha
213616	H70324	H70325	alpha
236027	H62070		alpha
248153	N53829	N73325	alpha
24991	(T80528)	R39000	alpha
26298	R13508	(R20629)	alpha
265817	N28661	N21457	alpha
266628		N22757	alpha
27342		R37173	alpha
280344	(N50305)	N47094	alpha
290894		N72005	alpha
294142		N68597	alpha
307787	W21278		alpha
340635	W56712	W56757	alpha
340683	W55988	W56276	alpha
346647	W94390	W74638	alpha
346796	W79585	W79784	alpha
359349	AA010546	AA010547	alpha
364632	AA022809	AA022690	alpha
39915		R50455	beta
40764	R56327	R56245	alpha
45086	H08908	H08824	alpha
46607	H10267	H10213	alpha
49811	H29080	H28976	alpha
50202		H17962	beta
50470		H16811	beta
66473	R16018	R16119	alpha
667794	AA258686	AA258608	alpha
69907	T48654	T48655	alpha
72391	AA394097	AA293803	gamma
739009	AA421586		beta
739014	(AA42185)	AA421567	beta
771303		AA443638	gamma
2-4		L36675	alpha
2-5		L36674	alpha
c-01f06		F01363	alpha
c-1rb08	F03254	F06981	alpha
c-2td12	F08836	F11169	alpha
c-28f08	F03751	F07521	alpha
cDNA	S69965		beta
EST01420 (HRBAA27)	M79265		gamma
EST19193	AA317129		beta
EST22040	AA319774		alpha

Figure 7/2

EST26845	T28079		beta
EST31489	AA328063		alpha
EST68G11	W22518		gamma
F1-625D	R29481		alpha
GEN-129D09	D81090		beta
hbc590	T11070		alpha
HIBBA65	T08213	T08212	alpha
	HR70E3R	HR70E3F	alpha
HSNACP0		U46896-46901	alpha
KK1311	N83633		alpha
		D318839	alpha
		L08850	alpha
	T28735		alpha
	Z20502		alpha

Figure 8


10 20 30 40 50 60 70
CCGCCGCAGCCGCCGCTCCATCCCCAGCCCCGGCCCCGCATCCGGTTTGGAAGGGGGCTGCAAGTTTGCA 70
AGGGGCCCGGGAXAAAAAXCGAGCAGTGGCCCTTCCCGCGTCCCCAGGGTTTCAAGGGACGCTAGGAXTX 140
TCCGCGGCCCTGGAGGTTCGCACTGGGGAGTGGGGTGAGATGGGGGGAAAGCGGGAGGGGGCTCAGGGTC 210
CAGAAGGGCXCCGCGGTCTCGGGAGTAGGGGGGCATXTGCGTCCCGCGGGAGGGGCTGGGGTGAGAGTGC 280
GGGGCCAGTGCACCGGTGCCCGTGTATCGCCCTCCCCAGGCCGCCAGGATGGACGTGTTCATGAAGGGCC 350
360 370 380 390 400 410 420
TGTCCATGGCCAAGGAGGGCGTTGTGGCAGCCGCGGAGAAAACCAAGCAGGGGGTCACCGAGGCGGCGGA 420
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710 720 730 740 750 760 770
AAAXCACACAXCCTCCTTTTTCTIATCTTTTTTACCATTATTAATAGTTATCTGGTGTTGAACACTTTCT 770
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1060 1070 1080 1090 1100 1110 1120
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CITCAGGTACTAGCCCAGCCCTGGCACCAGCCCTTCTCTCAMTTAGGCGGATGATCTGGCCGGGAACCAG 1400
1410 1420 1430 1440 1450 1460 1470
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2110 2120 2130 2140 2150 2160 2170
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Figure 9

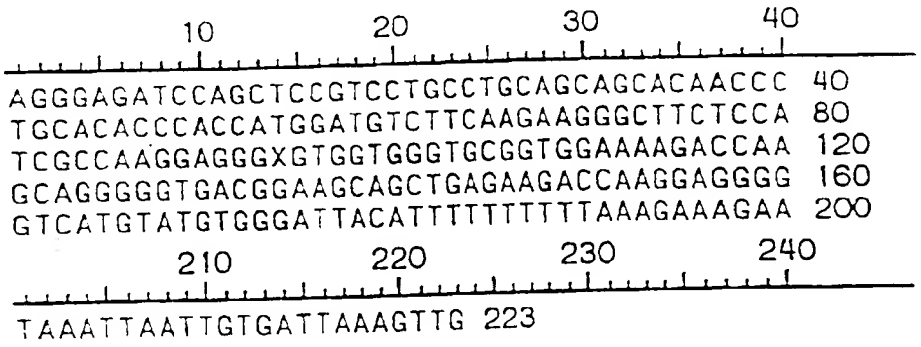


Figure 10

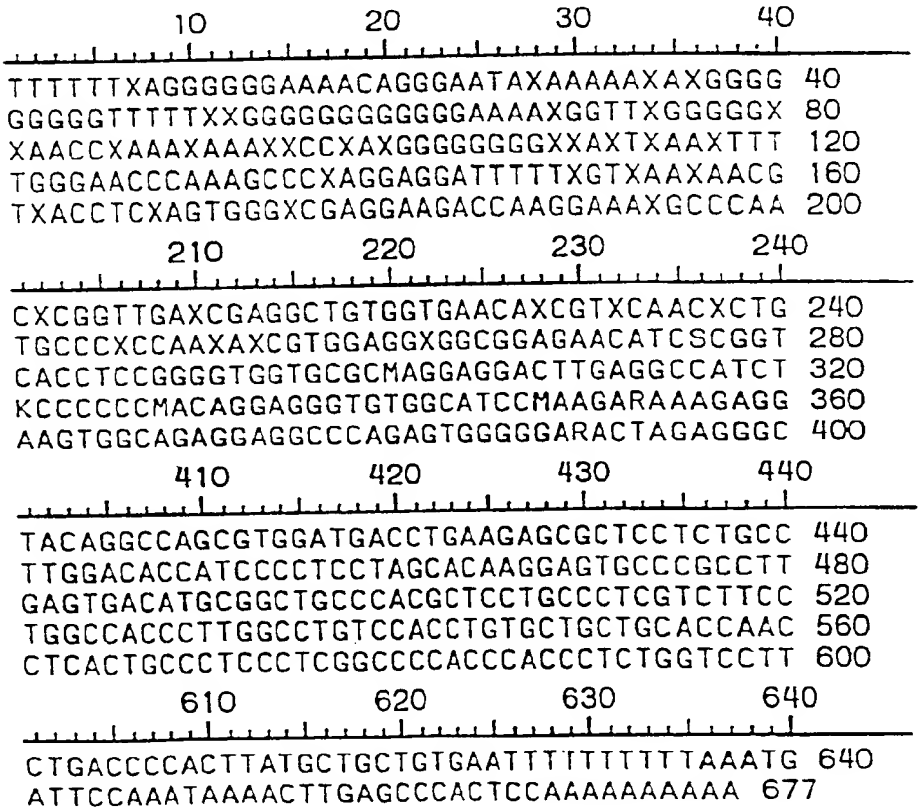


Figure 11/1

alpha-SYN exons 1-2

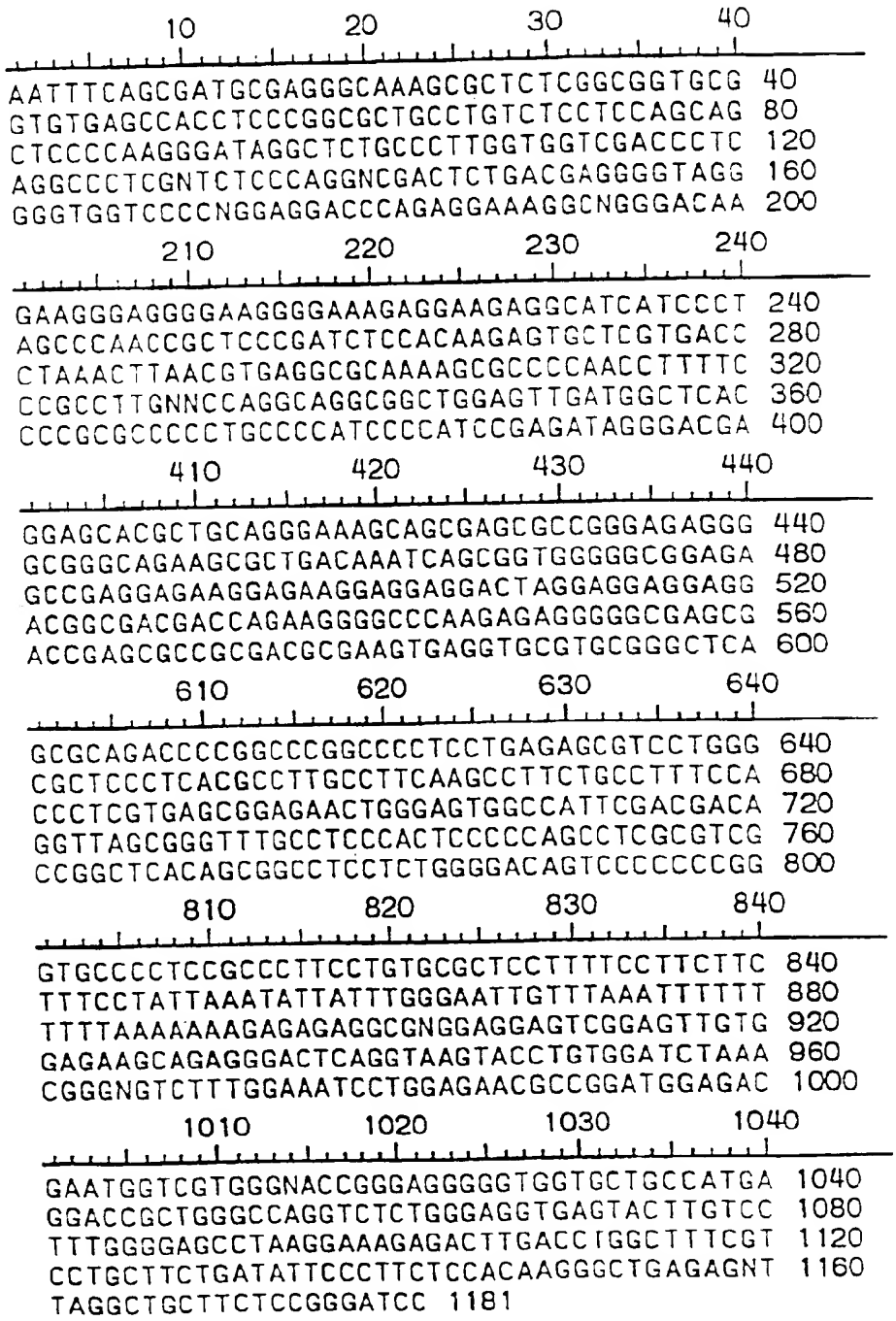


Figure 11/2

alpha-SYN exon 3

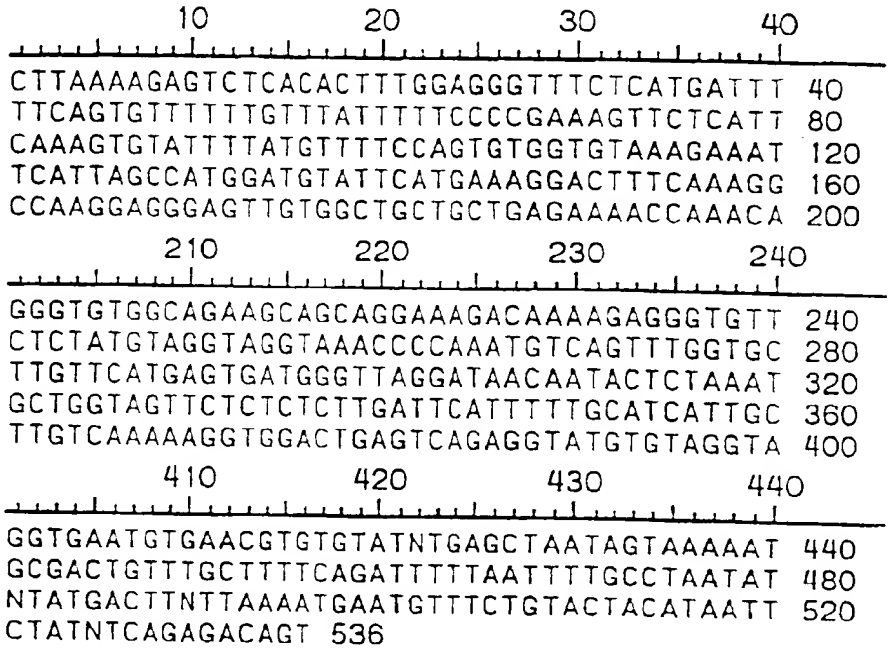


Figure 11/3

alpha-SYN exon 4

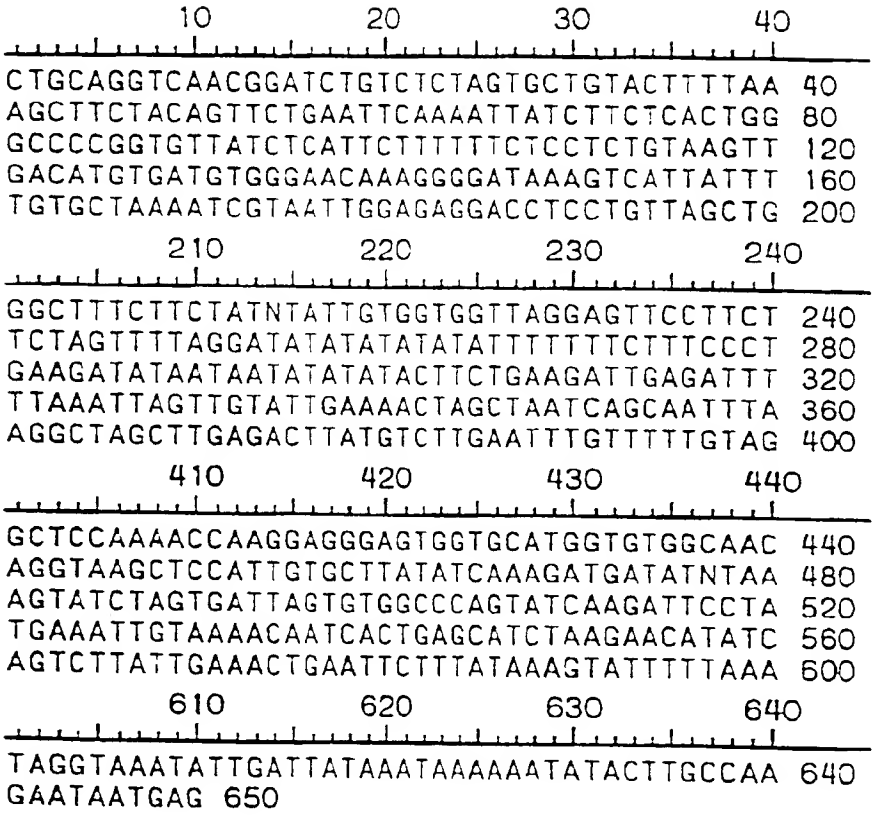


Figure 11/4

alpha-SYN exon 5

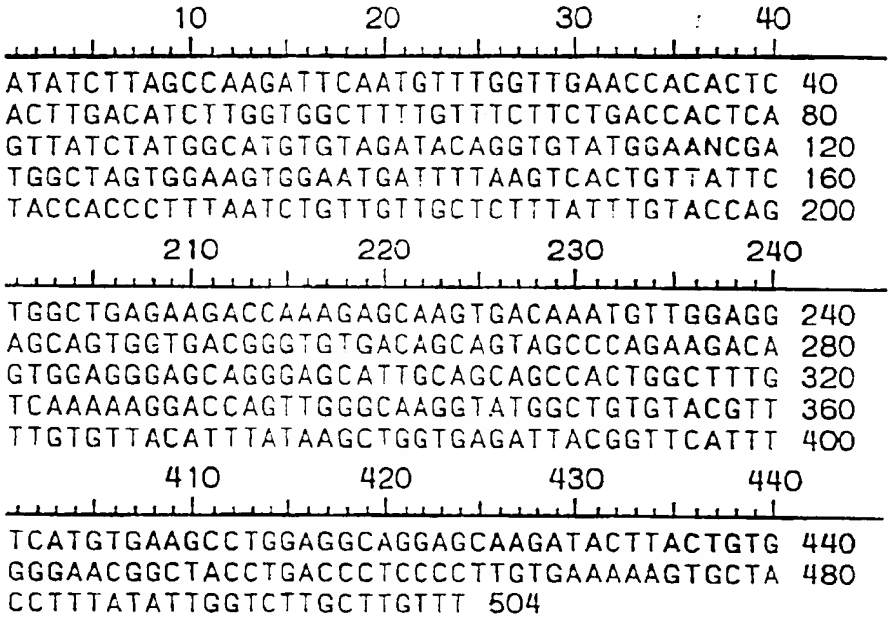
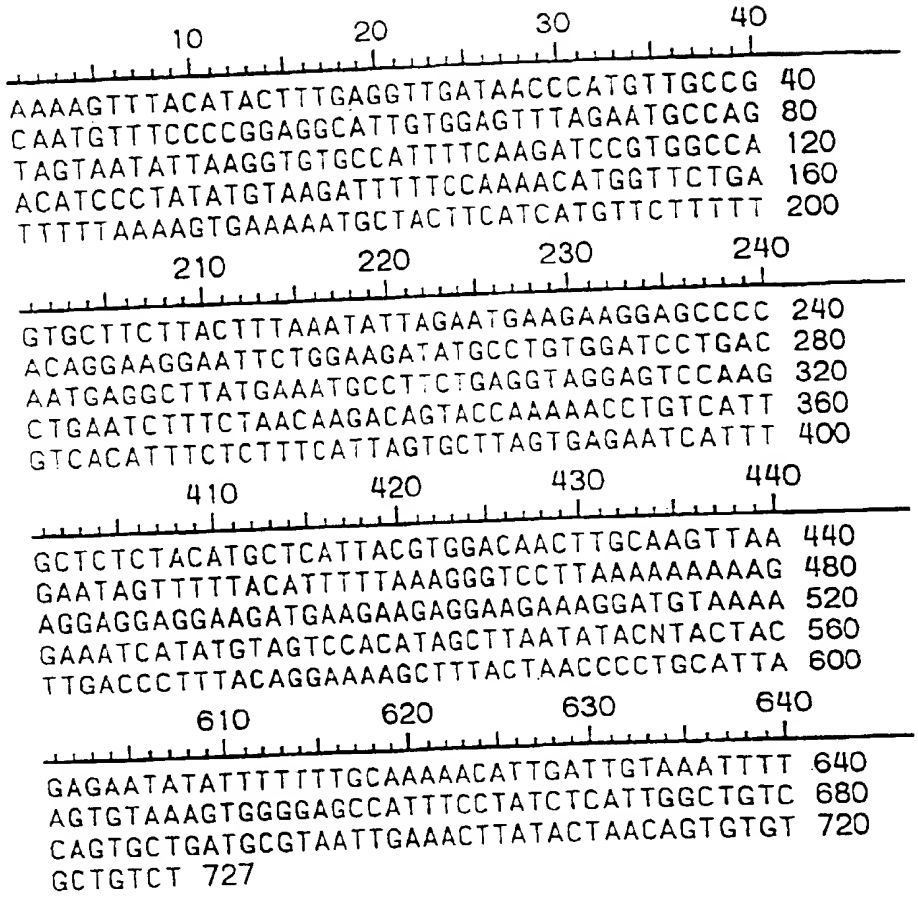


Figure 11/5

alpha-SYN exon 6



alpha-SYN exon 7

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TTCCAATGTGCCCAGTCATGACATTTCTCAAAGTTTTTAC 160
AGTGTATCTCGAAGTCTTCCATCAGCAGTGATTGAAGCAT 200
       210        220        230        240
CTGTACCTGCCCCCACTCAGCATTTCGGTGCTTCCCTTTC 240
ACTGAAGTGAATACATGGTAGCAGGGTCTTTGTGTGCTGT 280
GGATTTTGTGGCTTCAATCTACGATGTTAAAACAAATTAA 320
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ATTTTTTTGTTGCTGTTGTTCAGAAGTTGTTAGTGATTTG 400
       410        420        430        440
CTATCATATATTATNAGATTTTTAGGTGTCTTTTAATGAT 440
ACTGTCTAAGAATAATGACGTATTGTGAAATTTGTTAATA 480
TATATNATACTTAAAAATATGTGAGCATGAAACTATGCAC 520
CTATAATACTAAATATGAAATTTTACCATTTTGCGATGTG 560
TTTTATTCACTTGTGTTTGTATATNAATGGTGAGAATTAA 600
       610        620        630        640
AATAAAACGTTATCTCATTGCAAAAATATTTTATTTTTAT 640
CCCATCTCACTTTAATAATAAAAATCATGCTTATAAGCAA 680
CATGAATTAAGAACTGACACAAAGGACAAAAATATAAAGT 720
TATTAATAGCCATTTGAAGAAGGAGGAATTTTAGAAGAGG 760
TAGAGAAAATGGAACATTAACCCTACACTCGGAATTCCCT 800
       810        820        830        840
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TCCTTAAGTGGCTGTGATTAATTATTGAAAGTGGGGTGTT 880
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TGTTGTTTGATGTGTATGTGTTTATAATTGTTATACATTT 1000
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TTAATTGAGCCTTTTATTAACATATATTGTTATTTTTGTC 1040
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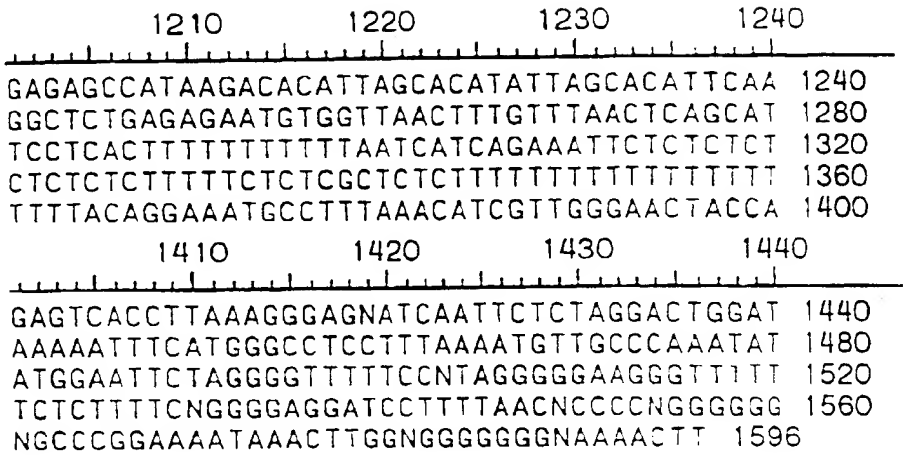
Figure 11/7

alpha-SYN exon 7



INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 98/13071

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 C12N15/11 C07K16/18 A61K48/00
C12Q1/68 G01N33/68 A01K67/027

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K C12Q G01N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	SCHAPIRA A. H.: "Pathogenesis of Parkinson's disease." BAILLERES CLINICAL NEUROLOGY, vol. 6, no. 1, April 1997, pages 15-36, XP002083889	1-23, 57-61,74
Y	see page 17, paragraph 2	24-56, 62-73
	see abstract	

Y	US 5 494 794 A (WALLACE DOUGLAS C) 27 February 1996 see the whole document	24-56, 62-73

	-/--	

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 November 1998	27/11/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl Fax: (+31-70) 340-3016	Mandl, B

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/13071

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JAKES R. ET AL.: "Identification of two distinct synucleins from human brain." FEBS LETTERS, vol. 345, 1994, pages 27-32, XP002078475 cited in the application & UEDA K. ET AL.: "Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease." PROC. NATL. ACAD. SCI. USA, vol. 90, 1993, pages 11282-11286, see figure 2 ---	1-74
A	CHEN X. ET AL: "The human NACP/alpha-synuclein gene: chromosome assignment to 4q21.3-q22 and TaqI RFLP analysis." GENOMICS, vol. 26, no. 2, 1995, pages 425-427, XP002083890 cited in the application ---	1-74
A	POLYMEROPOULOS M. H. ET AL.: "Mapping of a gene for Parkinson's disease to chromosome 4q21-q23." SCIENCE, vol. 274, 1996, pages 1197-1199, XP002083891 cited in the application see the whole document ---	1-74
A	MAROTEAUX L. AND SCHELLER R. H.: "The rat brain synucleins; family of proteins transiently associated with neuronal membrane." MOLECULAR BRAIN RESEARCH, vol. 11, 1991, pages 335-343, XP002083892 cited in the application see figure 1 ---	1-74
P,X	NUSSBAUM R. L. AND POLYMEROPOULOS M. H.: "Genetics of Parkinson's disease." HUMAN MOLECULAR GENETICS, vol. 6, no. 10, 1997, pages 1687-1691, XP002083893 see the whole document ---	1-74
P,X	GOEDERT M.: "The awakening of alpha-synuclein." NATURE, vol. 388, 17 July 1997, pages 232-233, XP002083894 see the whole document --- -/--	1-74

2

INTERNATIONAL SEARCH REPORT

Inter. onal Application No
PCT/US 98/13071

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No
P,X	POLYMEROPOULOS M. H. ET AL.: "Mutation in the alpha-synuclein gene identified in families with Parkinson's disease." SCIENCE, vol. 276, 27 June 1997, pages 2045-2047, XP002083895 see the whole document	1-74

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 98/13071

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5494794 A	27-02-1996	WO 9409162 A	28-04-1994